PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/47, 14/705, G01N 33/68, A61K 48/00, C07K 16/18

A1

(11) International Publication Number: WO 99/47562

(43) International Publication Date: 23 September 1999 (23.09.99)

(21) International Application Number: PCT/US99/05578

(22) International Filing Date: 16 March 1999 (16.03.99)

(30) Priority Data:
09/040,774 18 March 1998 (18.03.98) US

(71) Applicant: BIOSTRATUM, INC. [US/US]; Suite 120, 2605 Meridian Parkway, Durham, NC 27713 (US).

(72) Inventors: TRYGGVASON, Karl; Lokevagen 8 A, S–182 61 Djursholm (SE). KESTILA, Marjo; University of Oulu, Biocenter and Dept. of Biochemistry, FIN–90570 Linnama, Oulu (FI). LENKKERI, Ulla; University of Oulu, Biocenter and Dept. of Biochemistry, FIN–90570 Linnama, Oulu (FI). MANNIKKO, Minna; Jefferson Medical College, Dept. of Dermatology, 233 South 10th Street, Rm. 450 BLSB, Philadelphia, PA 19107 (US).

(74) Agent: HARPER, David, S.; McDonnell Boehnen Hulbert & Berghoff, 300 South Wacker Drive, Chicago, IL 60606 (US).

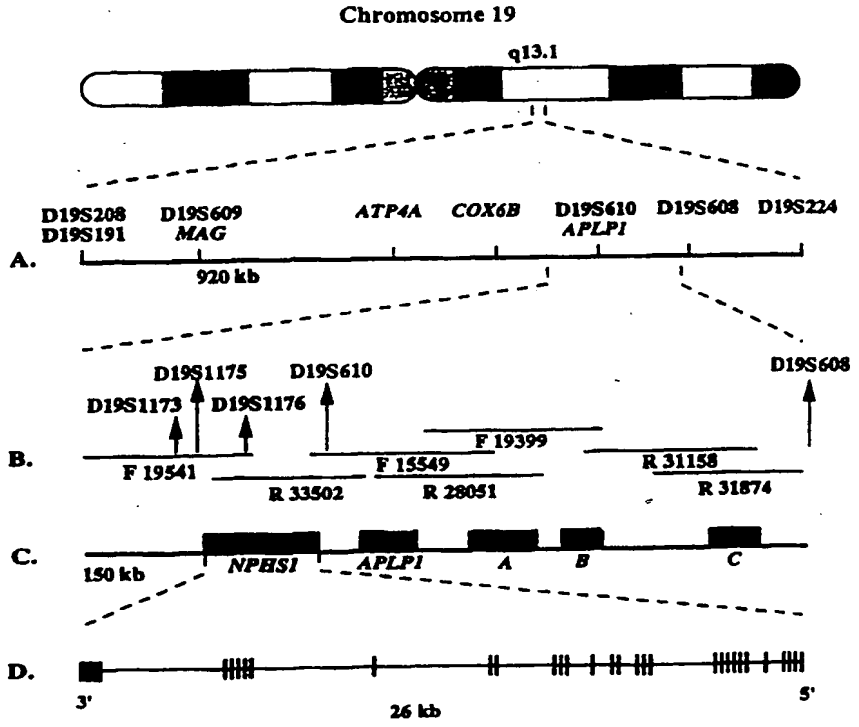
(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: *NEPHRIN* GENE AND PROTEIN

(57) Abstract

The present invention provides for compositions and methods for detecting susceptibility for basement membrane disease, in particular Congenital nephrotic syndromes of the Finnish type. The present invention for nucleic acids and protein for use in methods and compositions for the diagnosis of disease and identification of small molecule therapeutics for treatment of such disease, in particular of proteinuria associated with kidney disease.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Nephrin gene and protein

Cross Reference

This application claims priority from U.S. Patent Application Serial No. 09/040774 filed March 18, 1998.

Background of the Invention

Congenital nephrotic syndrome of the Finnish type (CNF, NPHS1, MIM 256300) is an autosomal recessive disorder, and a distinct entity among congenital nephrotic syndromes. It is characterized by massive proteinuria at the fetal stage and nephrosis at birth. Importantly, NPHS1 appears to solely affect the kidney and, therefore, it provides a unique model for studies on the glomerular filtration barrier.

The primary barrier for ultrafiltration of plasma in renal glomeruli comprises three layers; a fenestrated endothelium, a 300-350 nm thick glomerular basement membrane (GBM), and slit pores, i.e. diaphragms located between the foot processes of the epithelial cells. This barrier is a highly sophisticated size-selective molecular sieve whose molecular mechanisms of function are still largely unclarified. It is anticipated that the GBM, a tightly cross-linked meshwork of type IV collagen, laminin, nidogen and proteoglycans, contains pores that restrict the penetration of large proteins and cells, and, additionally, it has been hypothesized that anionic heparan sulfate proteoglycan components contribute to an electric barrier for macromolecules (Kasinath and Kanwar, 1993). The glomerular filter is affected in a large number of acquired and inherited diseases resulting in extensive leakage of plasma albumin and larger proteins leading to nephrotic syndrome and end stage

renal disease. Understanding of the molecular mechanisms of the glomerular filtration process and its pathology is of fundamental importance for clinical medicine, which, in turn, may facilitate novel developments for diagnosis and treatment of complications in primary and secondary diseases of the kidney. Genetic diseases with defects in the filtration barrier as major symptoms can serve as models for providing such knowledge.

Congenital nephrotic syndromes (NPHS) form a heterogenous group of diseases characterized by massive proteinuria at or shortly after birth (Rapola et al., 1992). Nephrotic syndrome can be primary, acquired, or a part of other syndromes. Congenital nephrotic syndrome of the Finnish type (CNF, NPHS1) is a distinct entity among NPHS. It is an autosomal recessive disorder with an incidence of 1:10,000 births in Finland, but considerably less in other countries (Norio, 1966; Huttunen, 1976). The disease manifests itself already at the fetal stage with heavy proteinuria *in utero*, demonstrating early lesions of the glomerular filtration barrier. The pathogenesis of NPHS1 has remained obscure. There are no pathognomonic pathologic features, the most typical histological finding of NPHS1 kidneys being dilation of the proximal tubuli (Huttunen *et al.* 1980). The kidneys are also large and have been found to contain a higher amount of nephrons than age-matched controls (Tryggvason and Kouvalainen, 1975). Electron microscopy reveals no abnormal features of the GBM itself, although there is a loss of foot processes of the glomerular epithelial cells, a finding characteristic for nephrotic syndromes of any cause. Analyses of GBM proteins, such as type IV collagen, laminin, and heparan sulfate proteoglycan have not revealed abnormal findings in NPHS1 (e.g. see Ljungberg et al. 1993, Kestilä et al. 1994a). NPHS1 is a progressive disease, usually

leading to death during the first two years of life, the only life-saving treatment being kidney transplantation (Holmberg et al. 1995). Importantly, most transplanted patients have, thus far, not developed extrarenal complications, suggesting that the mutated gene product is highly specific for kidney development and/or glomerular filtration function. However, about 20 % of the patients have developed post-transplantation nephrosis the cause of which is unknown (Laine et al., 1993; Holmberg et al., 1995).

Due to its high specificity for the glomerular filtration process, NPHS1 provides a unique model disease for studies on this important kidney function. Since there was no strong candidate gene for the disease, we have used the positional cloning approach in our attempts to identify the CNF gene, and have localised the gene to a 150 kb region on chromosome 19q13.1 (Kestilä et al., 1994b; Männikkö et al., 1995). We have identified a novel gene in the critical region and shown it to be mutated in NPHS1. The gene product is a novel transmembrane protein, which in the human embryo shows a high expression level in renal glomeruli.

Summary of the Invention

The present invention provides for the novel protein *Nephrin* and the gene encoding for this protein. The present invention encompasses a novel DNA nucleic acid sequence which is the nucleic acid sequence of SEQ ID NO:1 which encodes for the nephrin protein. The present invention also encompasses the protein encoded for by the coding regions of the nucleic acid sequence of SEQ ID NO:1 which has the amino acid sequence of SEQ ID NO:2. In particular, the present invention also

encompasses the mature nephrin protein in which the signal peptide has been cleaved off.

The present invention encompasses method, reagents and kits for screening individuals for the presence of mutated *Nephrin* gene for diagnosis, pre-natal screening, or post-natal screening for susceptibility to glomerular nephrosis or basement membrane disease. In particular, the present invention provides for screening for congenital nephrotic syndromes of the Finnish type (NPHS1).

The present invention provides for methods, reagents and kits for the therapeutic treatment of basement membrane disease associated with defective endogenous *Nephrin* gene product. Thus the present invention provides for therapeutic treatment using *Nephrin* protein, and in particular using protein produced by recombinant DNA methods. In addition, the present invention provides for gene therapy using therapeutic nucleic acid constructs containing the *Nephrin* gene, or substantially similar DNA sequence thereto.

Brief Description of the Drawings

The invention will be better understood in view of the attached drawings wherein:

Figure 1 is a drawing showing a physical map of the NPHS1 locus at 19q13.1 and genomic organisation of the *NPHS1* gene. Figure 1A, is a physical map of the 920 kb region between markers D19S208 and D19S224. Figure 1B, is a diagram of overlapping cosmid clones spanning the 150 kb critical region containing the *NPHS1* gene. Location of polymorphic markers are indicated by arrows. Figure 1C, is a diagram showing the location of five genes, *NPHS1*, *APLP1*, *A*, *B*, *C*,

characterised and searched for mutations in this study. Figure 1D, is a drawing showing a schematic structure of the *NPHS1* gene;

Figure 2 shows a northern blot analysis of nephrin expression (the *NPHS1* gene product) with mRNA from human embryonic and adult tissues. The northern filters containing 2 ug of human poly(A) RNA from four fetal and eight adult tissues (Clontech) were hybridized with a 1,371 bp nephrin cDNA probe (exons 1-10) made by RT-PCR from fetal kidney poly(A) RNA. Figure 2A, shows distinct expression can be seen only with fetal kidney RNA (arrow). Figure 2B, shows results using RNA from adult tissues, intense signal is only observed in a 4.3 kb band with kidney RNA (arrow), the other tissues exhibiting only insignificant if any positive signals. The tissues studied are marked above the filter and molecular size markers (kb) are shown to the sides of the filters;

Figure 3 is a diagram of Mutation analysis of the *NPHS1* gene. Left: (A) Pedigree of an NPHS1 family with an affected child having a 2-bp deletion in exon 2. Sequences of the deletion point shown from patient (homozygous), parent (heterozygous) and a healthy sibling. Right: (B) Pedigree of an NPHS1 family with an affected child having a nonsense mutation in exon 26. Sequences of the mutated region are shown from patient (homozygous), parent (heterozygous) and a healthy sibling;

Figure 4 is a diagram of the Nucleotide-derived amino acid sequence of nephrin (the *NPHS1* gene product) and predicted domain structure. Figure 4A, is the predicted N-terminal signal sequence is 22 residues, the cleavage site being marked with an arrow. A putative transmembrane domain (spanning residues 1,059-1086) is shown in bold and underlined. The putative extracellular part of the protein contains eight Ig-like modules (boxed), and one fibronectin type III -like module adjacent to the

transmembrane domain (boxed with a bold line, residues 941-1025). Cysteine residues are indicated by black dots and the ten putative N-glycosylation sites in the extracellular part of the protein are underlined. Figure 4 B shows the predicted domain structure of normal nephrin and the predicted effects of the two mutations (Fin-major and Fin-minor) identified in this study. The Ig-like modules are depicted by partial circles and the fibronectin type III like-motif by a hexagon. The transmembrane domain is shown as a black rectangle located in a membrane lipid bilayer. The locations of two free cysteine residues are indicated by lines with a black dot at the end. The Fin-major mutation would result in the production of part of the signal peptide and a short nonsense sequence. The Fin-minor mutation would result in a nephrin molecule lacking a part of the cytosolic domain; and

Figure 5 shows the results of expression of nephrin mRNA in human embryonic kidney by in situ hybridization. Figure 5A, shows intense expression in glomeruli throughout the renal cortex, little if any specific expression being observed in other structures. (4x objective magnification). Figure 5B, is a view at higher magnification which reveals intense expression in the periphery of individual glomeruli (straight arrows), probably mainly in epithelial cells. No expression is observed in the Bowman's capsule (bent arrow), proximal tubuli (open arrows), or endothelial cells of vessel walls. (20x objective magnification).

Detailed Description of the Invention

Congenital nephrotic syndrome of the Finnish type (CNF, NPHS1, MIM 256300) is an autosomal recessive disorder, and a distinct entity among congenital nephrotic syndromes. It is characterized by massive proteinuria at the fetal stage

and nephrosis at birth. Importantly, NPHS1 appears to solely affect the kidney and, therefore, it provides a unique model for studies on the glomerular filtration barrier. The NPHS1 gene has been localized to 19q13.1, and in the present study linkage disequilibrium was used to narrow the critical region to 150 kilobases which were sequenced. At least 10 novel genes, and one encoding amyloid precursor like protein were identified in this region. Five of the genes, all of which showed some expression in kidney, were analyzed by sequencing all their 63 exons in NPHS1 patients. Two mutations, a 2-bp deletion in exon 2 and a single base change in exon 26, both leading to premature stop codons were found in a novel 29-exon gene. The mutations were found either as homozygous or compound heterozygous in 44 out of 49 patients, 4 patients having the 2 bp deletion in one allele, the other potential mutation still being unknown. None among controls was found homozygous or compound heterozygous for the mutations. The gene product, termed *nephrin*, is a 1,241-residue putative transmembrane protein of the immunoglobulin family of cell adhesion molecules which by northern and *in situ* hybridization was shown to be kidney glomerulus-specific. The results demonstrate a crucial role for *nephrin* in the development or function of the kidney filtration barrier.

The invention will be more clearly understood by examination of the following examples, which are meant by way of illustration and not limitation.

Example 1

Methods and procedures

Sequencing of cosmid clones

Isolation of cosmid clones spanning the region between D19S208 and D19S608 has been reported previously (Olsen et al., 1996). DNA of cosmid clones F19541, R33502, F15549, R28051, F19399, R31158 and R31874 was mechanically sheared by nebulization and fragments of 1000-2000 bp were isolated and subcloned into M13 phage, prior to random sequencing using ABI 377 automated DNA sequencers.

Analysis of sequence

In order to develop new microsatellite markers, repeat regions were searched from the sequence, and three of them (D19S1173, D19S1175, D19S1176) were found to be polymorphic. Homology comparisons were performed using BLASTX and BLASTN programs (Altschul et al., 1990). Prior to BLASTN analyses, the nucleotide sequence was filtered using CENSOR (Jurka et al., 1996) to mask out repeat regions like Alu sequences. Exon prediction was made using GRAIL II (Uberbacher and Mural, 1991), GENSCAN (Burge and Karlin, 1997), FGENEH and HEXON (Solovyeh et al., 1994) programs, and prediction of the protein structure was made using BLASTP (Altschul et al., 1990) and EXPASY molecular biology server (Appel et al., 1994). The mutation search was performed by comparing patient sequences to the normal genomic sequence using the FASTA program of the GCG package (Genetics Computer Group, 1996).

Isolation of cDNAs

cDNAs were generated by PCR from poly(A) RNA from different tissues using primers based on the exon sequences. The PCR fragments were sequenced and

used for screening of cDNA libraries. Marathon ready cDNA kits (Clontech Laboratories) were also used to characterize the 5' and 3' extremities of the cDNAs. Comparison of the cDNA and genomic sequences were made to establish the sizes of introns, as were intron sequences at acceptor and donor splice sites.

Southern and Northern blots and in situ hybridization analyses

For Southern analyses samples containing 10 μg of genomic DNA were digested with different restriction enzymes and electrophreses on 1 % agarose gels, transferred to nylon membranes and hybridized with the cDNA probe. In multiple-tissue northern analysis poly(A) RNAs from 8 adult and 4 fetal tissues were studied (Clontech). Hybridization was done in ExpressHyb buffer at 65° C using a cDNA clone containing exons 1- 10.

For *in situ* hybridization a fragment from the NPHS1 cDNA clone (corresponding to exon 10) was labeled with digoxigenin (Boehringer Mannheim), cut to about 150 base pair fragments by alkaline hydrolysis, and then used as a probe. Tissue sections of 7 μm from a 23-week human embryonic kidney were treated with 0.2M HCl, 0.1M triethanolamine buffer, pH 8.0, containing 0.25% (v/v) acetic anhydride and 100 μg/ml proteinase K. The sections were hybridized with the probe at 62° C for 16 h. After rinsing in 50% formamide and standard sodium citrate, the probe was immunologically detected with an antibody to digoxigenin conjugated to alkaline phosphate enzyme (Boehringer Mannheim). The color was developed with NBT and BCIP.

Mutation analysis

In this study we analyzed 49 Finnish NPHS1 patients, their parents and a total of 54 healthy siblings. The diagnosis of NPHS1 is based on severe proteinuria, a large placenta (>25 % of birth weight), nephrotic syndrome during the first weeks of life, and exclusion of other types of congenital nephrotic syndrome (Koskimies 1990). Additionally, samples from 83 control individuals were analysed.

The NPHS1 gene was analysed by PCR-amplifying and sequencing all exon regions from genomic DNA. The sequences of the primers for exon 2 were 5'GAGAAAGCCAGACAGACGCAG3' (5' UTR) and 5'AGCTTCCGC-TGGTGGCT3' (intron 2), and the sequences of the primers for exon 26 were 5'CTCGGGGAGACCCACCC3' (intron 23) and 5'CCTGATGCTAACGG-CAGGGC3' (intron 26). PCR reactions were performed in a total volume of 25 ul, containing 20 ng of template DNA, 1x AmpliTaq buffer (Perkin-Elmer), 0.2 mM of each nucleotide, 50 ng of primers and 0.5 U AmpliTaq Gold DNA polymerase. The reactions were carried out for 30 cycles with denaturation at 95° C for 1 min, annealing at 60° C for 1 min, and extension at 72° C for 1 min. In the first cycle denaturation was carried out for 12 min, and extension in the last cycle was for 8 min. PCR products were separated by 1.5 % agarose gel, sliced off and purified by the QiaexII system (Qiagen). The purified PCR product was sequenced using specific primers employing dRhodamine dye-terminator chemistry and an ABI377 automated sequencer (Perkin-Elmer).

When screening for the NPHS1 Fin-major mutation from parents, siblings and controls, a 100 bp PCR product containing the exon 2 deletion site was amplified using a radioactively end-labeled primer, and electrophoresed on 6 % polyacrylamide gels. The second NPHS1 Fin-minor mutation could be screened for

using a novel restriction site for *Dde*I. The 140 bp amplified PCR product was digested with *Dde*I and the products (140 bp or 90 bp + 50 bp) were separated on an agarose gel (1 % SeaKem agarose - 3 % NuSieve agarose).

In general, methods and procedures for performing molecular biological and biochemical techniques are known in the art and can be found in available texts and references, such as for example Sambrook et al., (1989) Molecular Cloning: a laboratory manual, 2nd edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY); Short Protocols in Molecular Biology, 2nd edition (edited by Ausubel et al., John Wiley & Sons, New York, 1992); Davis et al., (1986) Basic Methods in Molecular Biology (Elsevier, New York); Gene Expression Technology (edited by David Goeddel, Academic Press, San Diego, CA, 1991).

Example 2

Characterization of genes at the CNF locus

Following localisation of the *NPHS1* gene to 19q13.1, overlapping cosmid clones from the interval of interest between markers D19S208 and D19S224 were isolated (Männikkö et al. 1995; Olsen et al., 1996). Based on the significant linkage disequilibrium observed with D19S608 and D19S610, as well as the new microsatellite markers, D19S1173, D19S1175, and D19S1176, identified in this study, the *NPHS1* gene was fine-mapped between D19S1175 and D19S608, in close vicinity of D19S1176 and D19S610 (Fig. 1). Southern hybridization analyses of NPHS1 patient DNA with genomic clones did not reveal variations, suggesting that the NPHS1 mutations do not represent major genomic rearrangements. The 150 kb critical region was sequenced in its entirety, and the sequence was searched for

potential candidate genes using exon prediction programs and data base similarity searches. Based on those analyses, the critical region was estimated to include over 100 potential exons. Similarity searches revealed one previously known gene, i.e. *APLP1* encoding an amyloid precursor -like protein (Lenkkeri et al., in press) and eight distinct expressed sequence tags (ESTs). Together, the analyses indicated the presence of at least ten novel genes in the critical region.

Figure 1 illustrates a physical map of the NPHS1 locus at 19q13.1 and genomic organisation of the *NPHS1* gene. Figure 1A, Physical map of the 920 kb region between D19S208 and D19S224. Figure 1B, Overlapping cosmid clones spanning the 150 kb critical region containing the *NPHS1* gene. Location of polymorphic markers are indicated by arrows. Figure 1C, Location of five genes, *NPHS1*, *APLP1*, *A*, *B*, *C*, characterised and searched for mutations in this study. Figure 1D, Schematic structure of the *NPHS1* gene.

Using Grail and Genscan exon prediction programs and sequences from cDNAs, the exon/intron structures of five of the genes, *NPHS1* (Fig. 1), *APLP1*, *A*, *B*, and *C* (not shown) were determined. Although steady state transcript levels varied, northern analyses revealed expression of all the genes in kidney, and with the exception of *NPHS1*, also in other tissues. Therefore, none of them could be excluded as the *NPHS1* gene and all were subjected to mutation analysis.

Example 3

Identification of the NPHS1 gene

Haplotype analyses of NPHS1 chromosomes have revealed two major classes in Finnish patients (Männikkö et al., 1995; this study). The first one

containing haplotypes 1-1-1-6-g-2-8-9 and 1-1-1-6-g-6-4-2 (markers D19S1173, D19S1175, D19S1176, D19S610, RFLP of gene *B*, D19S608, D19S224, D19S220, respectively) is the most common one found in 78 % of Finnish NPHS1 chromosomes. The second haplotype class, 3-5-3-6-a-8-10-x, is found in 13 % of cases. The remaining 9 % of observed haplotypes show totally different allele combinations, and have been thought to represent other mutations. Two major haplotype classes could represent the same mutation, because they both share allele 6 of D19S610. However, the present results demonstrated that they represent two different mutations.

Since Southern hybridization analyses did not reveal any major gene rearrangements, mutations were searched by direct sequencing of PCR-amplified exon regions of, if necessary, all the genes of this region.

The 17 exon *APLP1* gene located distal to D19S610 did not show variations between patients and controls, and was excluded as the *NPHS1* gene (Lenkkeri et al., in press). Also, the novel genes *A*, *B* and *C*, containing 9, 5 and 3 exons, respectively, did not have sequence variants segregating with NPHS1, and could similarly be excluded as the *NPHS1* genes (data not shown). A fourth novel gene *(NPHS1)* located proximal to D19S610 encoding a transcript of about 4.3 kb was shown to be strongly expressed in human embryonic and adult kidneys, no clear signals above background being observed in other tissues (Fig. 2).

Figure 2 illustrates the results of Northern analysis of nephrin expression with mRNA from human embryonic and adult tissues. The northern filters containing 2 ug of human poly(A) RNA from four fetal and eight adult tissues (Clontech) were hybridized with a 1,371 bp nephrin cDNA probe (exons 1-10) made

by RT-PCR from fetal kidney poly(A) RNA. In Figure 2A, Distinct expression can be seen only with fetal kidney RNA (arrow). In Figure 2B, Using RNA from adult tissues, intense signal is only observed in a 4.3 kb band with kidney RNA (arrow), the other tissues exhibiting only insignificant if any positive signals. The tissues studied are marked above the filter and molecular size markers (kb) are shown to the sides of the filters.

Therefore, this gene was a strong candidate for *NPHS1*. Full-length cDNA for the transcript was constructed using fetal kidney poly(A) mRNA (Clontech) and PCR primers made based on the predicted exon structure. The gene was found to have a size of 26 kb and to contain 29 exons (Fig. 1).

Exon sequencing analyses revealed the presence of two major mutations in over 90 % of NPHS1 chromosomes (Fig. 3). Figure 3 illustrates mutation analysis of the *NPHS1* gene. Left: (A) Pedigree of a NPHS1 family with an affected child having a 2-bp deletion in exon 2. Sequences of the deletion point shown from patient (homozygous), parent (heterozygous) and a healthy sibling. Right: (B) Pedigree of a NPHS1 family with an affected child having a nonsense mutation in exon 26. Sequences of the mutated region are shown from patient (homozygous), parent (heterozygous) and a healthy sibling.

The first mutation, a 2-bp deletion in exon 2 causes a frameshift resulting in the generation of a stop codon within the same exon. This mutation was found in all NPHS1 chromosomes with the haplotype 1-1-1-6-g-2-8-9 and 1-1-1-6-g-6-4-2 (total of 76 chromosomes). One out of 83 control individuals was heterozygous for the Fin-major mutation. The second sequence variant found in the *NPHS1* gene was a nonsense mutation CGA->TGA in exon 26, present in patients with haplotype 3-5-3-

6-a-8-10-x (13 chromosomes), and three patients with different haplotypes. None of the parents, healthy siblings, or controls (total of 230 individuals) were homozygous or compound heterozygous for the two mutations identified here. Since the gene cloned in this study is the one involved in a hereditary nephrotic syndrome, we refer to it as *NPHS1* gene.

Out of 49 NPHS1 patients studied, 32 were homozygous for the 2-bp deletion in exon 2 (Fin-major), four were homozygous for the nonsense mutation in exon 26 (Fin-minor), and eight were compound heterozygotes. Four patients had the Fin-major mutation in one allele, the other potential mutation still being unknown. One patient had neither one of the two mutations.

Example 4

Characterization of the NPHS1 gene product

The cDNA-predicted amino acid sequence of the NPHS1 protein (*nephrin*) is 1,241 residues (Fig. 4), with a calculated molecular mass of 134,742 without posttranslational modifications.

Figure 4 shows Nucleotide-derived amino acid sequence of *nephrin* and predicted domain structure (the NPHS1 gene product). Figure 4A illustrates the predicted N-terminal signal sequence is 22 residues, the cleavage site being marked with an arrow. A putative transmembrane domain (residues 1,059-1086) is shown in bold and underlined. The putative extracellular part of the protein contains eight Ig-like modules (boxed), and one fibronectin type III -like module adjacent to the transmembrane domain (boxed with a bold line). Cysteine residues are indicated by black dots and the ten putative N-glycosylation sites in the extracellular part of the

protein are underlined. Figure 4B illustrates predicted domain structure of normal *nephrin* (the NPHS1 gene product) and the predicted effects of the two mutations (Fin-major and Fin-minor) identified in this study. The Ig-like modules are depicted by partial circles and the fibronectin type III like-motif by a hexagon. The transmembrane domain is shown as a black rectangle located in a membrane lipid bilayer. The locations of three free cysteine residues are indicated by lines with a black dot at the end. The major NPHS1 mutation would result in the production of a secreted protein containing only a part of the first Ig-like module. The Fin-minor mutation would result in a nephrin molecule lacking a part of the cytosolic domain.

Several similarity comparison and protein structure prediction programs predicted that the NPHS1 protein would be a transmembrane protein of the immunoglobulin superfamily. There is a tentative 22-residue-long N-terminal signal peptide, an extracellular domain containing eight immunoglobulin-like domains, one fibronectin type III domain-like module, followed by a single putative transmembrane domain -like sequence, and a cytosolic C-terminal end. In spite of the presence of known structural modules (Fig. 4), the sequence identity with corresponding domains of proteins in the data base was relatively low. The tentative extracellular portion of the protein contains ten NXS or NXT consensus triplets for N-glycosylation. Furthermore, there are seven SG doublets, that are potential attachment sites for heparan sulfate.

Northern hybridization analysis carried out with poly(A) mRNA from four human embryonic and eight adult tissues revealed a high steady state level of the *NPHS1* gene transcript in the kidney, but not notably in other tissues. (Fig. 2). *In situ* hybridization carried out on a kidney sample from a 23-week-old human embryo

revealed intense expression signals in the glomeruli (Fig. 5 A). At higher magnification (Fig. 5 B), the signals could be seen in the periphery of mature and developing glomeruli, while the central mesangial regions are negative. It is apparent that the positive cells are epithelial podocytes. No specific signals were obtained with the antisense control probe.

Figure 5 illustrates expression of *nephrin* mRNA in human embryonic kidney by *in situ* hybridization. Figure 5A shows intense expression is seen in glomeruli throughout the renal cortex, little if any specific expression being observed in other structures. (4x objective magnification). Figure 5B, Higher magnification reveals intense expression in the periphery of individual glomeruli (straight arrows), probably mainly in epithelial cells. No expression is observed in the Bowman's capsule (bent arrow), proximal tubuli (open arrows), or endothelial cells of vessel walls. (20x objective magnification).

Example 5

The NPHS1 gene and its gene product nephrin.

Several lines of evidence obtained in the present study show that we have positionally cloned the gene affected in congenital nephrotic syndrome of the Finnish type. First, the defective gene is located in the critical 150 kb region on chromosome 19q13.1 to which the gene has been localized using linkage disequilibrium analyses (Kestilä et al., 1994b; Männikkö et al., 1995; Kestilä et al. manuscript). Second, the two mutations identified in the study were shown to be present, either as homozygous or compound heterozygous mutations, in 44 out of 49 Finnish patients studied. Four of the remaining patients had the major mutation in

one allele, the mutation in the other allele being, as yet, unidentified. One patient who did not have either of the two mutations, has a unique haplotype and, therefore, probably carries a different mutation. Third, individuals homozygous or compound heterozygous for the mutations were not found in 230 control DNAs. Additional, indirect evidence was the strong and practically renal glomeruli-specific expression of the gene, which implies involvement of the gene product in glomerular development or function.

Identification of the NPHS1 gene

The present identification of the *NPHS1* gene demonstrates the power of linkage disequilibrium analysis and direct DNA sequencing in the positional cloning of disease genes containing small mutations. Here, linkage disequilibrium mapping (Hästbacka et al., 1994) which when used with DNA from individuals of a homogenous population, such as the isolated Finnish population (de la Chapelle, 1993), was utilized to localize the *NPHS1* gene to a 150 kb genomic segment. In order to find genes located in this region, the entire segment was first sequenced, and using a combination of exon prediction programs and homology comparison analyses we could construct remarkably accurate gene structures that were verified from cDNAs. These cDNAs could be isolated either with the use of EST clones or by using the predicted exon sequences to construct cDNAs by PCR from mRNA. In this manner we could quickly identify 11 genes within the 150 kb *NPHS1* containing genomic segment. Since none of the genes was an obvious candidate for NPHS1, and no major gene rearrangements, such as deletions, insertions or inversions, were found in patient DNAs, search for small mutations had to be initiated, if necessary,

in all the 11 genes. Having determined the exon and cDNA sequences for the genes, methods such as SSCP and DGGE, which are frequently used for identification of small mutations, were potential alternatives. However, our experience from the search for small mutations in Alport syndrome (Barker et al., 1990; Tryggvason, 1996) suggests that these methods can frequently yield false negatives. For example, SSCP analyses in quite large patient populations have revealed only a 35-50 % mutation detection rate (Kawai et al., 1996, Knebelmann et al. 1996, Renieri et al., 1996), while our direct sequencing of PCR-amplified exon regions has yielded over 80 % detection. We therefore decided to use direct sequencing of exon regions to find the NPHS1 mutations. Although we had to sequence numerous exons of several genes, this relatively soon resulted in the identification of two small mutations in one gene. We conclude that sequencing of even a large candidate gene region and direct sequencing of its genes is an attractive and, above all, reliable method to search for small mutations in positional cloning, particularly if only few mutations can be expected to be present.

Genetics of NPHS1

Crucial components in the successful positional cloning of the *NPHS1* gene were the small isolated population, good clinical records and equal, high quality health care system which made it possible to reliably collect family samples. A typical situation in population isolates is that close to 100 % of cases are caused by the same mutation, and this phenomenon can already be seen in haplotype analysis. Observed changes in the founder haplotype, caused by historical recombinations, can be used to restrict the critical chromosomal region to a short genomic segment.

Thus, differences in the major NPHS1 haplotype 1-1-1-6-g-2-8-9 enabled substantial narrowing of the interval, leading to the isolation of the *NPHS1* gene. The major NPHS1 mutation causes only 78 % of cases, in contrast to many other "Finnish diseases" with 95-98 % prevalence of major disease alleles (*e.g.* Ikonen et al., 1991). However, the two main NPHS1 mutations characterized in this study together represent 94 % of Finnish cases.

Congenital nephrotic syndrome of the Finnish type is enriched in the Finnish population, but several cases can be found worldwide. Considerable immigration from Finland to Minnesota has also caused the spread of NPHS1 to the USA (Norio 1966; Mahan et al., 1984). In addition, several CNF cases have been diagnosed in different European countries, and linkage studies have supported association of analyzed families to the same chromosome 19 locus (Fuchshuber et al., 1996).

The identification of the *NPHS1* gene and disease causing mutations have immediate clinical significance, as they have enabled the development of exact DNA-based diagnosis for NPHS1 and carrier screening. This is particularly important, as we have recently demonstrated that the screening method widely used in Finland for NPHS1 based on measurements of alpha-fetoprotein levels in amniotic fluid can lead to false positive results and subsequent abortions of healthy NPHS1 carriers (Männikkö et al., 1997).

Nephrin - a glomerulus-specific cell adhesion receptor

Due to the high association of expression and pathology with glomeruli, the proximal part of the nephron, we have named the *NPHS1* gene product nephrin. The role of nephrin remains unknown, but it is likely to be an adhesion receptor and a

signaling protein, as its domain structure resembles that of a large group of cell adhesion receptors belonging to the immunoglobulin superfamily (Brümmendott and Rathjen, 1994).

The Ig-like domains of nephrin are all of type C2 which is particularly found in proteins participating in cell-cell or cell-matrix interactions. Between the sixth and seventh Ig-like domains there is a spacer of about 130 residues containing an unpaired cysteine, and there is another unpaired cysteine in the fibronectin type III - like domain. Their SH groups could be involved in the formation of *cis* homo/heterodimers, participate in thioether or thioester bonds with unknown structures, or be buried within the domains, as suggested by Brümmendott and Rathjen (1994).

Data base searches revealed that the cytosolic domain that contains nine tyrosine residues of nephrin has no significant homology with other known proteins. However, sequence motifs surrounding tyrosines suggest that tyrosines 1176, 1192 and 1217 could become phosphorylated during ligand binding of nephrin (see, Songyang et al. 1993). In that case, binding sites for the SH2-domains of *Src*-family kinases, *Abl*-kinase, and an adaptor protein *Nck* might be created (tyrosines 1176 and 1192 are followed by the motif DEV, and tyrosine 1217 by DQV). The crucial role for the intracellular domain of *nephrin* is emphasized by the fact that the Fin-minor mutation which results in the loss of 132 out of 155 residues results in full blown NPHS1.

The pathogenesis of NPHS1 has been thought to primarily or secondarily involve the highly anionic glycosaminoglycans, as the content of such molecules that are considered important for the glomerular filtration process is reported to be

decreased in the GBM in proteinuria (Kasinath and Kanwar, 1993). It cannot be excluded that *nephrin* is a proteoglycan, as it has several SG consensus sites for heparan sultate side chains, including the triplet SGD which is the major attachment sequence for the three large heparan sulfate side chains in the basement membrane proteoglycan perlecan (Noonan et al., 1991; Kallunki and Tryggvason, 1992; Dolan et al., 1997). However, thus far no Ig-like receptors have been reported to contain glycosaminoglycans.

How does nephrin function and what is its role in glomerular function? A vast majority of similar receptors interact with other membrane proteins in a homo- or heterophilic manner. However, some of the receptors have been shown to interact with extracellular matrix (ECM) proteins. For example, the myelin-associated glycoprotein MAG whose extracellular domain contains five Ig-like domains, interacts with different types of collagens and glycosaminoglycans (Fahrig et al., 1987). Furthermore, the axonal glycoprotein F11 and the deleted in colorectal cancer (DCC) protein have both been shown to bind tenascins and netrins, respectively (Zisch et al., 1992; Pesheva et al., 1993; Keino-Masu, 1996). Since it is possible that nephrin either binds another membrane protein or a protein of the ECM, which in this case would be the GBM, it will be important to localize nephrin by immunoelectron microscopy before embarking on the search for a specific ligand.

Whatever its function, the *in situ* hybridization analyses strongly suggested that *nephrin* is produced in glomerular epithelial cells that form the foot processes partially covering the outside of the glomerular capillaries. The ultimate filtration barrier for plasma macromolecules is located in the diaphragm covering the slit pores between the foot processes. In NPHS1 and nephrotic syndromes of other

causes, fusion of the foot processes is a general finding, and the structure or function of the slit pores are somehow affected with proteinuria as a result. It is proposed that the plasma membrane protein nephrin is important for maintaining the integrity of the foot processes of glomerular epithelial cells, or is crucial for their anchorage to components of the GBM.

Conclusions

The identification of the *NPHS1* gene will immediately find applications for diagnosis of the disease. Studies on the gene product *nephrin,* a putative cell adhesion and signaling receptor, may also provide a key to new fundamental knowledge on the molecular mechanisms of glomerular filtration, which despite decades of research are still poorly understood. As abnormal function of the filtration barrier is a major complication in many clinically important kidney diseases, such as diabetic nephropathy, nephrotic syndromes and glomerulonephritides, the present work is likely to have a more general impact on clinical nephrology. Immediate questions relate to the developmental expression and location of the protein, which would require the generation of antibodies and nucleotide probes for studies in animal and cell culture systems.

Example 6

Genetic Screening for Basement Membrane Disease

With the identification and characterisation of nephrin as a critical component in basement membrane disease associated with glomerular nephropathy, it is now possible to screen individuals, both pre- and post-natal screening, for susceptibility for basement membrane disease by detecting mutated *nephrin* gene or

protein. Such information will be useful to medical practitioners for the future diagnosis of disease conditions in screened individuals, and for planning preventative measures for the possible containment of future disease. Such information will be useful for the diagnosis of currently active disease conditions. The present invention allows for the diagnosis of currently active disease conditions, as being related to basement membrane disease by detecting mutated *nephrin* gene or protein. The discovery of the *nephrin* gene provides a means for detecting the presence of the *nephrin* gene in individuals, and for the determination of the presence of any mutations in said gene. Such means for detection comprises nucleic acids having the entire *nephrin* gene sequence, or fragments thereof which will specifically hybridize to said *nephrin* gene, or mRNA transcripts from said nephrin gene under stringent conditions. An additional means for detection of the *nephrin* gene and mutations therein comprise specific contiguous fragments of said gene, and complementary gene sequence, which can be combined for use as primers for amplifying the targeted gene sequence. Said means for detection of mutations in a *nephrin* gene also comprise direct hybridization of normal gene with target gene and subsequent detection of successful hybridization. In all cases, the target gene may be amplified or unamplified DNA or RNA isolated from the individual to be tested.

Antibody Screening of Tissues and Samples

By having the *NPHS1* gene sequence, it is well within the skill of one in the art to use existing molecular biology and biochemical techniques to construct and use an expression vector which will produce recombinant nephrin protein, or fusion protein, purify this protein, and produce antibodies specifically reactive with

nephrin. The expression of proteins in bacterial, yeast, insect and mammalian cells is known in the art. It is known in the art how to construct and use expression vectors in which the expressed gene contains one or more introns. The production of monoclonal antibodies is well known in the art, and the use of polyclonal and monoclonal antibodies for immunohistochemical detection of protein in tissue samples is a routine practice. A wide variety of detectable labels are available for use in immunohistochemical staining and immunoassays for detection of protein in samples such as homogenised tissue, blood, serum, urine or other bodily fluids.

One of ordinary skill in the art will be able to readily use the teachings of the present invention to design suitable assays and detection schemes for practising the screening methods contemplated by the present invention.

Gene Therapy

Given the teaching of the present invention, it will be possible to address deficiencies in *Nephrin* gene or protein by gene therapy or therapy using recombinant protein. Methods for the administration of protein and gene therapy are known in the art.

GenBank Accession Numbers

The accession numbers for the cosmid clones characterised are: F19541 = U95090, R33502 = AC002133, R28051 = AD000864, F19399 = AD000833, R31158 = AD000827, R31874 = AD000823. The accession for the *nephrin* cDNA sequence is AF035835.

One of ordinary skill in the art will be able to readily use the teachings of the present invention to design and construct suitable nucleic acid sequences which will be the functional equivalents of those disclosed. One of ordinary skill in the art will know that there exisits many allelic variants of the disclosed nucleic acid sequnences which still encode for a nephrin protein with equivalent function. The teaching of the present invention allows for the discovery of mutations in the nephrin gene and the modified protein therein encoded.

Example 7

Screening for Small Molecule Therapeutics

With the identification and characterisation of nephrin as a critical component in kidney pathothogy and proteinuria, and thus implicated in many kidney diseases, it is now possible to screen for small molecule therapeutics using nephrin and the neprhin gene. Screening for such therapeutics can be accomplished by sequential selective screening for activity and molecules which specifically hybridize to nephrin, or which specifically effect the expression of the neprhrin gene. Selective screening can be performed on pools of small molecule compounds generated by standard combinatorial chemistry, on known moleucles, or in combination with computer modeling of the nephrin protein structure and rational drug design. Such methods and techniques are known in the art.

Literature Cited

Altschul, S. F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. J. Mol. Biol. 215, 403-10

Appel, R.D., Bairoch, A. and Hochstrasser, D.F. (1994) A new generation of information retrieval tools for biologists: the example of the ExPASy WWW server. Trends Biochem. Sci. 19: 258-260

Barker, D., Hostikka, S.L., Zhou, J., Chow, L.T., Oliphant, A.R., Gerken, S.C., Gregory, M.C., Skolnick, M.H., Atkin, C.L. and Tryggvason, K. (1990) Identification of mutations in the COL4A5 collagen gene in Alport syndrome. Science 248, 1224-1227

Brümmendott, T., and Rathjen, F.G. (1994) Cell adhesion molecules 1: Immunoglobulin superfamily. Protein profile 1, 951-1058.

Burge, C. and Karlin, S. (1997) Prediction of complete gene structures in human genomic DNA. J. Mol. Biol. 268, 78-94.

de la Chapelle, A. (1993) Disease gene mapping in isolated human populations: the example of Finland. J. Med. Genet. 30:857-865

Dolan, M., Horchar, T., Rigatti, B., and Hassell, J.R. (1997) Identification of sites in domain I perlecan that regulate heparan sulfate synthesis. J. Biol. Chem. 272, 4316-4322

Fahrig, T., Landa, C., Pesheva, P., Kühn, K., and Schacher, M. (1987) Characterization of binding properties of the myelin-associated glycoprotein to extracellular matrix constituents. EMBO J. 6, 2875-2883.

Fuchshuber, A., Niaudet, P., Gribouval, O., Genevieve, J., Gubler, M-C., Broyer, M. and Antignac, C. (1996) Congenital nephrotic syndrome of the Finnish type: linkage to the locus in a non-Finnish population. Pediatr. Nephrol. 10: 135-138

Genetics Computer Group, Program manual for the Wisconsin Package, Version 9, December 1996, 575 Science Drive, Madison, Wisconsin, USA 53711

Hästbacka, J., de la Chapelle, A., Mahtani, M.M., Clines, G., Reeve-Daly, M.P., Daly, M., Hamilton, B.A., Kusumi, K., Trivedi, B., Weaver, A., Coloma, A., Lovett, M., Buckler, A., Kaitila, I., and Lander, E.S. (1994) The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. Cell 78, 1073-1087

Holmberg, C., Antikainen, M., Rönnholm, K., Ala-Houhala, M. and Jalanko, H. (1995) Management of congenital nephrotic syndrome of the Finnish type. Pediatr. Nephrol. 9: 87-93

Huttunen, N.P., Rapola, J., Vilska, J. and Hallman, N. (1980) Renal pathology in congenital nephrotic syndrome of the Finnish type: a quantitative light microscopic study on 50 patients. Int. J. Pediatr. Nephr. 1: 10-16

Ikonen, E., Baumann, M., Grön, K., Syvänen, A-C., Enomaa, N., Halila, R., Aula, P. and Peltonen, L. (1991) Aspartylglucosaminuria: cDNA encoding human aspartylglucosaminidase and the missense mutation causing the disease. EMBO J. 10: 51-58

Jurka, J., Klonowski, P., Dagman, V., Pelton, P. (1996) CENSOR - a program for identification and elimination of repetitive elements from DNA sequences. Computers and Chemistry Vol. 20 (No. 1): 119-122.

Kallunki, P., and Tryggvason, K. (1992) Human basement membrane heparan sulfate proteoglycan core protein: a 467 kD protein containing multiple domains resembling elements of the low density lipoprotein receptor, laminin, neural cell adhesion molecules, and epidermal growth factor. J. Cell Biol. 116, 559-571

Kasinath, B.S. and Kanwar, Y.S. (1993) Glomerular basement membrane: biology and physiology. In: Molecular and cellular aspects of basement membranes (D. Rorhbach and R. Timpl, eds), Academic Press, pp. 89-106.

Kawai, S., Nomura, S., Harano, T., Fukushima, T., & Osawa, G.(1996) The COL4A5 gene in Japanese Alport syndrome patients: spectrum of mutations of all exons. Kidney Int. 49, 814-822

Keino-Masu, K., Masu, M. Hinck, L., Leonardo, E.D., Chan, S.S.Y., Culotti, J.G., and Tessier-Lavigne, M. (1996) Cell 87, 175-185

Kestilä, M., Männikkö, M., Holmberg, C., Korpela, K., Savolainen, E.R., Peltonen, L. and Tryggvason, K. (1994a) Exclusion of eight genes as mutated loci in congenital nephrotic syndrome of the Finnish type. Kidney Int. 45, 986-990

Kestilä, M., Männikkö, M., Holmberg, C., Gyapay, G., Weissenbcah, J., Savolainen, E.R., Peltonen, L. and Tryggvason, K. (1994b) Congenital nephrotic syndrome of the Finnish type maps to the long arm of chromosome 19. Am. J. Hum. Genet. 54, 757-764

Knebelmann, B., Breillat, C., Forestier, L., Arrondel, C., Jacassier, D., et al. (1996) Spectrum of mutations in the COL4A5 collagen gene in X-linked Alport syndrome. Am. J., Hum. Genet. 59, 1221-1232

Koskimies, O. (1990) Genetics of congenital and early infantile nephrotic syndromes. In: Spitzer, A., Avner, E.D. (eds) Inheritance of kidney and uritary tract diseases. Kluwer, Boston, Dordrecht and London, p. 131-138

Laine, J., Jalanko, H., Holthöfer, H., Krogerus, L., Rapola, J., von Willebrand, E., Lautenschlager, I., Salmela, K. and Holmberg, C. (1993) Post-transplantation

nephrosis in congenital nephrotic syndrome of the Finnish type. Kidney Int. 44: 867-874

Lenkkeri, U., Kestilä, M., Lamerdin, J., McCready, P., Adamson, A., Olsen, A. and Tryggvason, K. Structure of the human amyloid precursor like protein gene APLP1 at 19q13.1. Human Genetics, in press

Ljungberg, P., Jalanko, H., Holmberg, C. and Holthöfer, H. (1993) Congenital nephrosis of the Finnish type (CNF): matrix components of the glomerular basement membranes and of cultured mesangial cells. Histochem. J. 25: 606-612

Mahan, J.D., Mauer, S.M. Sibley, R.K. and Vernier, R.L. (1984) Congenital nephrotic syndrome: Evolution of medical management and results of renal transplantation. J. Pediatr. 105: 549-557

Männikkö, M., Kestilä, M., Holmberg, C., Norio, R., Ryynänen, M., Olsen, A., Peltonen, L. and Tryggvason, K. (1995) Fine mapping and haplotype analysis of the locus for congenital nephrotic syndrome on chromosome 19q13.1. Am. J. Hum. Genet. 57: 1377-1383

Männikkö, M., Kestilä, M., Lenkkeri, U., Alakurtti, H., Holmberg, C., Leisti, J., Salonen, R., Aula, P., Mustonen, A., Peltonen, L. and Tryggvason, K. (1997) Improved prenatal diagnosis of the congenital nephrotic syndrome of the Finnish type nased on DNA analysis, Kidney Int. 51: 868-872

Martin, P., Heiskari, N., Hertz, J.-M-, Atkin, C., Barker, D., et al. Survey of mutations in the COL4A5 collagen gene in patients with suspected Alport syndrome: PCR amplification and direct sequencing of all 51 exon regions reveals over 80 mutation detection rate. (manuscript).

Noonan, D. M., Fulle, A., Valente, P., Cai, S., Horigan, E., Sasaki, M., Yamada, Y., and Hassell, J.R. (1991) The complete sequence of perlecan, a basement membrane heparan sulfate proteoglycan, reveals extensive similarity with laminin A chain, low density lipoprotein receptor, and the neural cell adhesion molecule. J. Biol. Chem. 266, 22939-22947

Norio, R. (1966) Heredity on the congenital nephrotic syndrome. Ann. Paediatr. Fenn. 12 (suppl 27):1-94

Olsen, A., Georgescu, A., Johnson, S. and Carrano, A.V. (1996) Assembly of a 1-Mb restriction-mapped cosmid contig spanning the candidate region for Finnish congenital nephrosis (NPHS1) in 19q13.1. Genomics 34:223-225

Pesheva, P., Gennarini, G., Goridis, C., and Schacher, M. (1993) The F3/11 cell adhesion molecule mediates the repulsion of neurons by the extracellular matrix glycoprotein J1-160/180. Neuron 10, 69-82

Pekkarinen P., Kestilä M., Hakola P., Järvi O., Tryggvason, K. and Palotie L. Fine-scale mapping of the PLO-SL locus. (manuscript)

Rapola, J., Huttunen, N.P. and Hallman, N. (1992) Congenital and infantile nephrotic syndrome. In: Edelman CM (ed.) Pediatric Kidney Disease. 2nd ed. Little, Brown and Company, Boston. Vol 2: 1291-1305

Renieri, A., Bruttini, M., Galli, L., Zanelli, P., Neri, T., et al. (1996) X-linked Alport syndrome: an SSCP-based mutation survey over all 51 exons of the COL4A5 gene. Am. J. Hum. Genet. 58, 1192-1204

Solovyev, V.V., Salamov, A.A., Lawrence, C.B. (1994) Predicting internal exons by oligonucleotide composition and discriminant analysis of spliceable open reading frames. Nucl. Acids Res. 22(24): 5156-5163

Tryggvason, K. (1996) Mutations in type IV collagen genes in Alport syndrome. In: Molecular pathology and Genetics of Alport syndrome (ed. K. Tryggvason). Contrib. Nephrol., 117, 154-171, Karger, Basel

Tryggvason, K. and Kouvalainen, K. (1975) Number of nephrons in normal human kidneys and kidneys of patients with the congenital nephrotic syndrome. Nephron 15: 62-68

Uberbacher, E.C. and Mural, R.J. (1991) Locating protein-coding regions in human DNA sequences by a multiple sensor-neural network approach, Proc. Natl Acad. Sci. USA 88: 11261-11265

Zisch, A.H., D'Allessandri, L., Ranscht, B., Falchetto, R., Winterhalter, K.H., and Vaughan, L. (1992) Neuronal cell adhesion molecule contactin/F11 binds to tenascin via its immunoglobulin-like domains. J. Cell Biol. 119, 203-213

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Tryggvason, Karl
Kestila, Marjo
Lenkkeri, Ulla
Mannikko, Minna

(ii) TITLE OF INVENTION: Nephrin Gene and Protein

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: McDonnell Boehnen Hulbert & Berghoff
(B) STREET: 300 S. Wacker Drive, Suite 3200
(C) CITY: Chicago
(D) STATE: IL
(E) COUNTRY: USA
(F) ZIP: 60606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: To be assigned
(B) FILING DATE: Herewith
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Harper, David
(B) REGISTRATION NUMBER: 42,636
(C) REFERENCE/DOCKET NUMBER: 97,842-B

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (312)913-0001
(B) TELEFAX: (312)913-0002

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4285 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..66
(D) OTHER INFORMATION: /note= "putative signal peptide"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..3723

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 67..3723

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 121..122
(D) OTHER INFORMATION: /note= "deletion mutation FIN-Major"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3800..3804
(D) OTHER INFORMATION: /note= "nonsense mutation in exon 26 FIN-Minor"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3178..3258
(D) OTHER INFORMATION: /note= "putative transmembrane domain"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG GCC CTG GGG ACG ACG CTC AGG GCT TCT CTC CTG CTC CTG GGG CTG
48
Met Ala Leu Gly Thr Thr Leu Arg Ala Ser Leu Leu Leu Leu Gly Leu
-22      -20                 -15                 -10

CTG ACT GAA GGC CTG GCG CAG TTG GCG ATT CCT GCC TCC GTT CCC CGG
96
Leu Thr Glu Gly Leu Ala Gln Leu Ala Ile Pro Ala Ser Val Pro Arg
     -5                   1                   5                  10

GGC TTC TGG GCC CTG CCT GAA AAC CTG ACG GTG GTG GAG GGG GCC TCA
144
Gly Phe Trp Ala Leu Pro Glu Asn Leu Thr Val Val Glu Gly Ala Ser
                  15                  20                  25

GTG GAG CTG CGT TGT GGG GTC AGC ACC CCT GGC AGT GCG GTG CAA TGG
192
Val Glu Leu Arg Cys Gly Val Ser Thr Pro Gly Ser Ala Val Gln Trp
              30                  35                  40

GCC AAA GAT GGG CTG CTC CTG GGC CCC GAC CCC AGG ATC CCA GGC TTC
240
```

```
Ala Lys Asp Gly Leu Leu Leu Gly Pro Asp Pro Arg Ile Pro Gly Phe
          45                  50                  55

CCG AGG TAC CGC CTG GAA GGG GAC CCT GCT AGA GGT GAA TTC CAC CTG
288
Pro Arg Tyr Arg Leu Glu Gly Asp Pro Ala Arg Gly Glu Phe His Leu
      60                  65                  70

CAC ATC GAG GCC TGT GAC CTC AGC GAT GAC GCG GAG TAT GAG TGC CAG
336
His Ile Glu Ala Cys Asp Leu Ser Asp Asp Ala Glu Tyr Glu Cys Gln
 75                  80                  85                  90

GTC GGC CGC TCT GAG ATG GGG CCC GAG CTC GTG TCT CCC AGA GTG ATC
384
Val Gly Arg Ser Glu Met Gly Pro Glu Leu Val Ser Pro Arg Val Ile
                95                 100                 105

CTC TCC ATC CTG GTT CCT CCC AAG CTG CTC CTG CTG ACC CCA GAG GCA
432
Leu Ser Ile Leu Val Pro Pro Lys Leu Leu Leu Leu Thr Pro Glu Ala
            110                 115                 120

GGC ACC ATG GTC ACC TGG GTA GCT GGG CAG GAG TAC GTG GTC AAC TGT
480
Gly Thr Met Val Thr Trp Val Ala Gly Gln Glu Tyr Val Val Asn Cys
        125                 130                 135

GTG TCT GGG GAC GCG AAG CCA GCA CCT GAC ATC ACC ATT CTC CTG AGT
528
Val Ser Gly Asp Ala Lys Pro Ala Pro Asp Ile Thr Ile Leu Leu Ser
    140                 145                 150

GGA CAG ACA ATA TCT GAC ATC TCT GCA AAC GTG AAC GAG GGC TCC CAG
576
Gly Gln Thr Ile Ser Asp Ile Ser Ala Asn Val Asn Glu Gly Ser Gln
155                 160                 165                 170

CAG AAA CTC TTC ACT GTG GAG GCC ACA GCC AGG GTG ACA CCC CGG AGC
624
Gln Lys Leu Phe Thr Val Glu Ala Thr Ala Arg Val Thr Pro Arg Ser
                175                 180                 185

TCA GAT AAT AGG CAG TTG CTG GTC TGT GAG GCG TCT AGC CCA GCA CTG
672
Ser Asp Asn Arg Gln Leu Leu Val Cys Glu Ala Ser Ser Pro Ala Leu
            190                 195                 200

GAG GCC CCC ATC AAG GCC TCA TTC ACC GTG AAT GTT CTG TTC CCT CCA
720
Glu Ala Pro Ile Lys Ala Ser Phe Thr Val Asn Val Leu Phe Pro Pro
        205                 210                 215

GGA CCC CCT GTC ATC GAG TGG CCA GGC CTG GAT GAG GGG CAC GTG CGG
768
Gly Pro Pro Val Ile Glu Trp Pro Gly Leu Asp Glu Gly His Val Arg
    220                 225                 230
```

```
GCA GGA CAG AGC TTG GAG CTG CCG TGC GTG GCC CGA GGG GGT AAT CCC
816
Ala Gly Gln Ser Leu Glu Leu Pro Cys Val Ala Arg Gly Gly Asn Pro
235                 240                 245                 250

TTA GCC ACA CTG CAG TGG CTG AAG AAT GGC CAG CCG GTG TCC ACA GCG
864
Leu Ala Thr Leu Gln Trp Leu Lys Asn Gly Gln Pro Val Ser Thr Ala
                255                 260                 265

TGG GGC ACA GAG CAC ACC CAG GCG GTG GCC CGC AGT GTG CTG GTG ATG
912
Trp Gly Thr Glu His Thr Gln Ala Val Ala Arg Ser Val Leu Val Met
            270                 275                 280

ACC GTG AGG CCA GAA GAC CAT GGA GCG CAG CTC AGC TGC GAG GCC CAC
960
Thr Val Arg Pro Glu Asp His Gly Ala Gln Leu Ser Cys Glu Ala His
        285                 290                 295
AAC AGC GTG TCT GCA GGG ACC CAG GAG CAC GGC ATC ACA CTG CAG GTC
1008
Asn Ser Val Ser Ala Gly Thr Gln Glu His Gly Ile Thr Leu Gln Val
    300                 305                 310

ACC TTT CCC CCT AGT GCC ATT ATT ATC TTG GGA TCT GCA TCC CAG ACT
1056
Thr Phe Pro Pro Ser Ala Ile Ile Ile Leu Gly Ser Ala Ser Gln Thr
315                 320                 325                 330

GAG AAC AAG AAC GTG ACA CTC TCC TGT GTC AGC AAG TCC AGT CGC CCG
1104
Glu Asn Lys Asn Val Thr Leu Ser Cys Val Ser Lys Ser Ser Arg Pro
                335                 340                 345

CGG GTT CTG CTA CGA TGG TGG CTG GGC TGG CGG CAG CTG CTG CCC ATG
1152
Arg Val Leu Leu Arg Trp Trp Leu Gly Trp Arg Gln Leu Leu Pro Met
            350                 355                 360

GAG GAG ACA GTC ATG GAT GGA CTG CAT GGC GGT CAC ATC TCC ATG TCC
1200
Glu Glu Thr Val Met Asp Gly Leu His Gly Gly His Ile Ser Met Ser
        365                 370                 375

AAC CTG ACA TTC CTG GCG CGG CGG GAG GAC AAC GGT CTG ACC CTC ACA
1248
Asn Leu Thr Phe Leu Ala Arg Arg Glu Asp Asn Gly Leu Thr Leu Thr
    380                 385                 390

TGT GAG GCC TTC AGT GAA GCC TTC ACC AAG GAG ACC TTC AAG AAG TCG
1296
Cys Glu Ala Phe Ser Glu Ala Phe Thr Lys Glu Thr Phe Lys Lys Ser
395                 400                 405                 410

CTC ATC CTG AAC GTA AAA TAT CCC GCC CAG AAA CTG TGG ATT GAG GGT
1344
```

```
Leu Ile Leu Asn Val Lys Tyr Pro Ala Gln Lys Leu Trp Ile Glu Gly
                415                 420                 425

CCC CCA GAG GGC CAG AAG CTC CGG GCT GGG ACC CGG GTG AGG CTG GTG
1392
Pro Pro Glu Gly Gln Lys Leu Arg Ala Gly Thr Arg Val Arg Leu Val
            430                 435                 440

TGT TTG GCT ATC GGG GGC AAC CCA GAG CCC TCC CTC ATG TGG TAC AAG
1440
Cys Leu Ala Ile Gly Gly Asn Pro Glu Pro Ser Leu Met Trp Tyr Lys
        445                 450                 455

GAC TCG CGC ACC GTG ACC GAG TCG CGG CTG CCG CAG GAG TCG CGG CGC
1488
Asp Ser Arg Thr Val Thr Glu Ser Arg Leu Pro Gln Glu Ser Arg Arg
    460                 465                 470

GTG CAT CTC GGC AGC GTG GAG AAA TCT GGG AGC ACC TTC TCC CGA GAG
1536
Val His Leu Gly Ser Val Glu Lys Ser Gly Ser Thr Phe Ser Arg Glu
475                 480                 485                 490

CTG GTG CTG GTC ACA GGG CCG TCG GAC AAC CAG GCC AAG TTC ACG TGC
1584
Leu Val Leu Val Thr Gly Pro Ser Asp Asn Gln Ala Lys Phe Thr Cys
                495                 500                 505

AAG GCT GGA CAG CTC AGC GCG TCC ACG CAG CTG GCG GTG CAG TTT CCC
1632
Lys Ala Gly Gln Leu Ser Ala Ser Thr Gln Leu Ala Val Gln Phe Pro
            510                 515                 520

CCA ACT AAC GTG ACG ATC CTG GCC AAC GCA TCC GCA CTG CGC CCG GGA
1680
Pro Thr Asn Val Thr Ile Leu Ala Asn Ala Ser Ala Leu Arg Pro Gly
        525                 530                 535

GAC GCC TTA AAC TTG ACA TGC GTC AGC GTC AGC AGC AAT CCG CCG GTC
1728
Asp Ala Leu Asn Leu Thr Cys Val Ser Val Ser Ser Asn Pro Pro Val
    540                 545                 550

AAC TTG TCC TGG GAC AAG GAA GGG GAG AGG CTG GAG GGC GTG GCC GCC
1776
Asn Leu Ser Trp Asp Lys Glu Gly Glu Arg Leu Glu Gly Val Ala Ala
555                 560                 565                 570

CCA CCC CGG AGA GCC CCA TTC AAA GGC TCC GCC GCC GCC AGG AGC GTC
1824
Pro Pro Arg Arg Ala Pro Phe Lys Gly Ser Ala Ala Ala Arg Ser Val
                575                 580                 585

CTT CTG CAA GTG TCA TCC CGC GAT CAT GGC CAG CGC GTG ACC TGC CGC
1872
Leu Leu Gln Val Ser Ser Arg Asp His Gly Gln Arg Val Thr Cys Arg
```

590 595 600

GCC CAC AGC GCC GAG CTC CGC GAA ACC GTG AGC TCC TTC TAT CGC CTC
1920
Ala His Ser Ala Glu Leu Arg Glu Thr Val Ser Ser Phe Tyr Arg Leu
605 610 615

AAC GTA CTG TAC CGT CCA GAG TTC CTG GGG GAG CAG GTG CTG GTG GTG
1968
Asn Val Leu Tyr Arg Pro Glu Phe Leu Gly Glu Gln Val Leu Val Val
620 625 630

ACC GCG GTG GAG CAG GGC GAG GCG TTG CTG CCC GTG TCC GTG TCC GCT
2016
Thr Ala Val Glu Gln Gly Glu Ala Leu Leu Pro Val Ser Val Ser Ala
635 640 645 650

AAC CCC GCC CCC GAG GCC TTC AAC TGG ACC TTC CGC GGC TAT CGC CTC
2064
Asn Pro Ala Pro Glu Ala Phe Asn Trp Thr Phe Arg Gly Tyr Arg Leu
655 660 665

AGT CCA GCG GGC GGC CCC CGG CAT CGC ATC CTG TCC AGC GGG GCT CTG
2112
Ser Pro Ala Gly Gly Pro Arg His Arg Ile Leu Ser Ser Gly Ala Leu
670 675 680

CAT CTG TGG AAT GTG ACC CGC GCG GAC GAC GGC CTC TAT CAG CTG CAC
2160
His Leu Trp Asn Val Thr Arg Ala Asp Asp Gly Leu Tyr Gln Leu His
685 690 695

TGC CAG AAC TCT GAG GGC ACC GCG GAA GCG CGG CTG CGG CTG GAC GTG
2208
Cys Gln Asn Ser Glu Gly Thr Ala Glu Ala Arg Leu Arg Leu Asp Val
700 705 710

CAC TAT GCT CCC ACC ATC CGT GCC CTC CAG GAC CCC ACT GAG GTG AAC
2256
His Tyr Ala Pro Thr Ile Arg Ala Leu Gln Asp Pro Thr Glu Val Asn
715 720 725 730

GTC GGG GGT TCT GTG GAC ATA GTC TGC ACT GTC GAT GCC AAT CCC ATC
2304
Val Gly Gly Ser Val Asp Ile Val Cys Thr Val Asp Ala Asn Pro Ile
735 740 745

CTC CCG GGC ATG TTC AAC TGG GAG AGA CTG GGA GAA GAT GAG GAG GAC
2352
Leu Pro Gly Met Phe Asn Trp Glu Arg Leu Gly Glu Asp Glu Glu Asp
750 755 760

CAG AGC CTG GAT GAC ATG GAG AAG ATA TCC AGG GGA CCA ACG GGG CGC
2400
Gln Ser Leu Asp Asp Met Glu Lys Ile Ser Arg Gly Pro Thr Gly Arg
765 770 775

```
CTG CGG ATT CAC CAT GCC AAA CTG GCC CAG GCT GGC GCT TAC CAG TGC
2448
Leu Arg Ile His His Ala Lys Leu Ala Gln Ala Gly Ala Tyr Gln Cys
    780                 785                 790

ATT GTG GAC AAT GGG GTG GCG CCT CCA GCA CGA CGG CTG CTC CGT CTT
2496
Ile Val Asp Asn Gly Val Ala Pro Pro Ala Arg Arg Leu Leu Arg Leu
795                 800                 805                 810

GTT GTC AGA TTT GCC CCC CAG GTG GAG CAC CCC ACT CCC CTA ACT AAG
2544
Val Val Arg Phe Ala Pro Gln Val Glu His Pro Thr Pro Leu Thr Lys
                815                 820                 825

GTG GCT GCA GCT GGA GAC AGC ACC AGT TCT GCC ACC CTC CAC TGC CGT
2592
Val Ala Ala Ala Gly Asp Ser Thr Ser Ser Ala Thr Leu His Cys Arg
            830                 835                 840

GCC CGA GGT GTC CCC AAC ATC GTT TTC ACT TGG ACA AAA AAC GGG GTC
2640
Ala Arg Gly Val Pro Asn Ile Val Phe Thr Trp Thr Lys Asn Gly Val
        845                 850                 855

CCT CTG GAT CTC CAA GAT CCC AGG TAC ACG GAG CAC ACA TAC CAC CAG
2688
Pro Leu Asp Leu Gln Asp Pro Arg Tyr Thr Glu His Thr Tyr His Gln
    860                 865                 870

GGT GGT GTC CAC AGC AGC CTC CTG ACC ATT GCC AAC GTG TCT GCC GCC
2736
Gly Gly Val His Ser Ser Leu Leu Thr Ile Ala Asn Val Ser Ala Ala
875                 880                 885                 890

CAG GAT TAC GCC CTC TTC ACA TGT ACA GCC ACC AAC GCC CTT GGC TCG
2784
Gln Asp Tyr Ala Leu Phe Thr Cys Thr Ala Thr Asn Ala Leu Gly Ser
                895                 900                 905

GAC CAA ACC AAC ATT CAA CTT GTC AGC ATC AGC CGC CCT GAC CCT CCA
2832
Asp Gln Thr Asn Ile Gln Leu Val Ser Ile Ser Arg Pro Asp Pro Pro
            910                 915                 920

TCA GGA TTA AAG GTT GTG AGT CTG ACC CCA CAC TCC GTG GGG CTG GAG
2880
Ser Gly Leu Lys Val Val Ser Leu Thr Pro His Ser Val Gly Leu Glu
        925                 930                 935

TGG AAG CCT GGC TTT GAT GGG GGC CTG CCA CAG AGG TTC TGC ATC AGG
2928
Trp Lys Pro Gly Phe Asp Gly Gly Leu Pro Gln Arg Phe Cys Ile Arg
    940                 945                 950
```

```
TAT GAG GCC CTG GGG ACT CCA GGG TTC CAC TAT GTG GAT GTC GTA CCA
2976
Tyr Glu Ala Leu Gly Thr Pro Gly Phe His Tyr Val Asp Val Val Pro
955                 960                 965                 970

CCC CAG GCC ACC ACC TTC ACG CTG ACT GGT CTA CAG CCT TCT ACA AGA
3024
Pro Gln Ala Thr Thr Phe Thr Leu Thr Gly Leu Gln Pro Ser Thr Arg
                975                 980                 985

TAC AGG GTC TGG CTG CTG GCC AGT AAT GCC TTG GGG GAC AGT GGA CTG
3072
Tyr Arg Val Trp Leu Leu Ala Ser Asn Ala Leu Gly Asp Ser Gly Leu
            990                 995                1000

GCT GAC AAA GGG ACC CAG CTT CCC ATC ACT ACC CCA GGT CTC CAC CAG
3120
Ala Asp Lys Gly Thr Gln Leu Pro Ile Thr Thr Pro Gly Leu His Gln
        1005                1010                1015

CCT TCT GGA GAA CCT GAA GAC CAG CTG CCC ACA GAG CCA CCT TCA GGA
3168
Pro Ser Gly Glu Pro Glu Asp Gln Leu Pro Thr Glu Pro Pro Ser Gly
    1020                1025                1030

CCC TCG GGG CTG CCC CTG CTG CCT GTG CTG TTC GCT CTT GGG GGG CTT
3216
Pro Ser Gly Leu Pro Leu Leu Pro Val Leu Phe Ala Leu Gly Gly Leu
1035                1040                1045                1050

CTG CTC CTC TCC AAT GCC TCC TGT GTC GGG GGG GTC CTC TGG CAG CGG
3264
Leu Leu Leu Ser Asn Ala Ser Cys Val Gly Gly Val Leu Trp Gln Arg
                1055                1060                1065

AGA CTC AGG CGT CTT GCT GAG GGC ATC TCA GAG AAG ACA GAG GCA GGG
3312
Arg Leu Arg Arg Leu Ala Glu Gly Ile Ser Glu Lys Thr Glu Ala Gly
            1070                1075                1080

TCG GAA GAG GAC CGA GTC AGG AAC GAA TAT GAG GAG AGC CAG TGG ACA
3360
Ser Glu Glu Asp Arg Val Arg Asn Glu Tyr Glu Glu Ser Gln Trp Thr
        1085                1090                1095

GGA GAG CGG GAC ACT CAG AGC TCC ACG GTC AGC ACA ACA GAG GCA GAG
3408
Gly Glu Arg Asp Thr Gln Ser Ser Thr Val Ser Thr Thr Glu Ala Glu
    1100                1105                1110

CCG TAT TAC CGC TCC CTG AGG GAC TTC AGC CCC CAG CTG CCC CCG ACG
3456
Pro Tyr Tyr Arg Ser Leu Arg Asp Phe Ser Pro Gln Leu Pro Pro Thr
1115                1120                1125                1130
```

```
CAG GAG GAG GTG TCT TAT TCC CGA GGT TTC ACA GGT GAA GAT GAG GAT
3504
Gln Glu Glu Val Ser Tyr Ser Arg Gly Phe Thr Gly Glu Asp Glu Asp
                1135                1140                1145

ATG GCC TTC CCT GGG CAC TTG TAT GAT GAG GTA GAA AGA ACG TAC CCC
3552
Met Ala Phe Pro Gly His Leu Tyr Asp Glu Val Glu Arg Thr Tyr Pro
            1150                1155                1160

CCG TCT GGA GCC TGG GGA CCC CTC TAC GAT GAA GTG CAG ATG GGA CCC
3600
Pro Ser Gly Ala Trp Gly Pro Leu Tyr Asp Glu Val Gln Met Gly Pro
        1165                1170                1175

TGG GAC CTC CAC TGG CCT GAA GAC ACA TAT CAG GAT CCA AGA GGA ATC
3648
Trp Asp Leu His Trp Pro Glu Asp Thr Tyr Gln Asp Pro Arg Gly Ile
    1180                1185                1190

TAT GAC CAG GTG GCC GGA GAC TTG GAC ACT CTG GAA CCC GAT TCT CTG
3696
Tyr Asp Gln Val Ala Gly Asp Leu Asp Thr Leu Glu Pro Asp Ser Leu
1195                1200                1205                1210

CCC TTC GAG CTG AGG GGA CAT CTG GTG TAAGAGCCCT CTCAACCCCA
3743
Pro Phe Glu Leu Arg Gly His Leu Val
                1215

TTGTCCTGCA CCTGCAGGAA TTTACACTCC ACTGGTCTCT CTCATTACAG CCTGGGCCGA
3803

GCTGGTTAGG TGAGCTCCAT AAAACCCAAA GGGACTTGGT GTCAGGAGAG GACATGGAGG
3863

GGGCTGAGTG ACAGAGATGG TTCAGCTGGT ACCAGAGTAG AAACAAGGTG CATCCTGGGG
3923

TTGGCTTTAG AAACTAAACT TCTCCAAAAG GACAGGGCAG ATTGTAAACG TCGTCTCAAA
3983

AATGAAATGC TGCCGGGTGC GGTGACTCAC GCCTATAATC CCAGCACTTT GGGAGGCTGA
4043

GGCGGGTGGA TCACCTGAGG TCAGGAGTTC GAGACCAGCC TGGCCAACAT GGTAAAACTC
4103

CATTTCTACT AAAAATATAA AAAATTAGCC AGGAGTAGTG GCGCATGCCT GTAGTCCCAG
4163

CTACTTGGGA GGCTGATGCA TGAGAATTGC TTGAACCCAG GAGGCGGAGG TTGCAGTGAG
4223

CTGAGATCAC GCCACTGCAC TCCAGCCTGG GCGACAGAGC GAGATTCTGT CTCAAAAAAT
4283
```

AA
4285

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1241 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ala Leu Gly Thr Thr Leu Arg Ala Ser Leu Leu Leu Leu Gly Leu
-22  -20                 -15                 -10

Leu Thr Glu Gly Leu Ala Gln Leu Ala Ile Pro Ala Ser Val Pro Arg
 -5                   1                   5                  10

Gly Phe Trp Ala Leu Pro Glu Asn Leu Thr Val Val Glu Gly Ala Ser
                15                  20                  25

Val Glu Leu Arg Cys Gly Val Ser Thr Pro Gly Ser Ala Val Gln Trp
            30                  35                  40

Ala Lys Asp Gly Leu Leu Leu Gly Pro Asp Pro Arg Ile Pro Gly Phe
        45                  50                  55

Pro Arg Tyr Arg Leu Glu Gly Asp Pro Ala Arg Gly Glu Phe His Leu
    60                  65                  70

His Ile Glu Ala Cys Asp Leu Ser Asp Asp Ala Glu Tyr Glu Cys Gln
75                  80                  85                  90

Val Gly Arg Ser Glu Met Gly Pro Glu Leu Val Ser Pro Arg Val Ile
                95                  100                 105

Leu Ser Ile Leu Val Pro Pro Lys Leu Leu Leu Leu Thr Pro Glu Ala
            110                 115                 120

Gly Thr Met Val Thr Trp Val Ala Gly Gln Glu Tyr Val Val Asn Cys
        125                 130                 135

Val Ser Gly Asp Ala Lys Pro Ala Pro Asp Ile Thr Ile Leu Leu Ser
    140                 145                 150

Gly Gln Thr Ile Ser Asp Ile Ser Ala Asn Val Asn Glu Gly Ser Gln
155                 160                 165                 170

Gln Lys Leu Phe Thr Val Glu Ala Thr Ala Arg Val Thr Pro Arg Ser
                175                 180                 185

Ser Asp Asn Arg Gln Leu Leu Val Cys Glu Ala Ser Ser Pro Ala Leu
            190                 195                 200
```

Glu Ala Pro Ile Lys Ala Ser Phe Thr Val Asn Val Leu Phe Pro Pro
205 210 215

Gly Pro Pro Val Ile Glu Trp Pro Gly Leu Asp Glu Gly His Val Arg
220 225 230

Ala Gly Gln Ser Leu Glu Leu Pro Cys Val Ala Arg Gly Gly Asn Pro
235 240 245 250

Leu Ala Thr Leu Gln Trp Leu Lys Asn Gly Gln Pro Val Ser Thr Ala
255 260 265

Trp Gly Thr Glu His Thr Gln Ala Val Ala Arg Ser Val Leu Val Met
270 275 280

Thr Val Arg Pro Glu Asp His Gly Ala Gln Leu Ser Cys Glu Ala His
285 290 295

Asn Ser Val Ser Ala Gly Thr Gln Glu His Gly Ile Thr Leu Gln Val
300 305 310

Thr Phe Pro Pro Ser Ala Ile Ile Ile Leu Gly Ser Ala Ser Gln Thr
315 320 325 330

Glu Asn Lys Asn Val Thr Leu Ser Cys Val Ser Lys Ser Ser Arg Pro
335 340 345

Arg Val Leu Leu Arg Trp Trp Leu Gly Trp Arg Gln Leu Leu Pro Met
350 355 360

Glu Glu Thr Val Met Asp Gly Leu His Gly Gly His Ile Ser Met Ser
365 370 375

Asn Leu Thr Phe Leu Ala Arg Arg Glu Asp Asn Gly Leu Thr Leu Thr
380 385 390

Cys Glu Ala Phe Ser Glu Ala Phe Thr Lys Glu Thr Phe Lys Lys Ser
395 400 405 410

Leu Ile Leu Asn Val Lys Tyr Pro Ala Gln Lys Leu Trp Ile Glu Gly
415 420 425

Pro Pro Glu Gly Gln Lys Leu Arg Ala Gly Thr Arg Val Arg Leu Val
430 435 440

Cys Leu Ala Ile Gly Gly Asn Pro Glu Pro Ser Leu Met Trp Tyr Lys
445 450 455

Asp Ser Arg Thr Val Thr Glu Ser Arg Leu Pro Gln Glu Ser Arg Arg
460 465 470

Val His Leu Gly Ser Val Glu Lys Ser Gly Ser Thr Phe Ser Arg Glu
475 480 485 490

Leu Val Leu Val Thr Gly Pro Ser Asp Asn Gln Ala Lys Phe Thr Cys
495 500 505

```
Lys Ala Gly Gln Leu Ser Ala Ser Thr Gln Leu Ala Val Gln Phe Pro
            510                 515                 520

Pro Thr Asn Val Thr Ile Leu Ala Asn Ala Ser Ala Leu Arg Pro Gly
        525                 530                 535

Asp Ala Leu Asn Leu Thr Cys Val Ser Val Ser Ser Asn Pro Pro Val
    540                 545                 550

Asn Leu Ser Trp Asp Lys Glu Gly Glu Arg Leu Glu Gly Val Ala Ala
555                 560                 565                 570

Pro Pro Arg Arg Ala Pro Phe Lys Gly Ser Ala Ala Ala Arg Ser Val
                575                 580                 585

Leu Leu Gln Val Ser Ser Arg Asp His Gly Gln Arg Val Thr Cys Arg
            590                 595                 600

Ala His Ser Ala Glu Leu Arg Glu Thr Val Ser Ser Phe Tyr Arg Leu
        605                 610                 615

Asn Val Leu Tyr Arg Pro Glu Phe Leu Gly Glu Gln Val Leu Val Val
    620                 625                 630

Thr Ala Val Glu Gln Gly Glu Ala Leu Leu Pro Val Ser Val Ser Ala
635                 640                 645                 650

Asn Pro Ala Pro Glu Ala Phe Asn Trp Thr Phe Arg Gly Tyr Arg Leu
                655                 660                 665

Ser Pro Ala Gly Gly Pro Arg His Arg Ile Leu Ser Ser Gly Ala Leu
            670                 675                 680

His Leu Trp Asn Val Thr Arg Ala Asp Asp Gly Leu Tyr Gln Leu His
        685                 690                 695

Cys Gln Asn Ser Glu Gly Thr Ala Glu Ala Arg Leu Arg Leu Asp Val
    700                 705                 710

His Tyr Ala Pro Thr Ile Arg Ala Leu Gln Asp Pro Thr Glu Val Asn
715                 720                 725                 730

Val Gly Gly Ser Val Asp Ile Val Cys Thr Val Asp Ala Asn Pro Ile
                735                 740                 745

Leu Pro Gly Met Phe Asn Trp Glu Arg Leu Gly Glu Asp Glu Glu Asp
            750                 755                 760

Gln Ser Leu Asp Asp Met Glu Lys Ile Ser Arg Gly Pro Thr Gly Arg
        765                 770                 775

Leu Arg Ile His His Ala Lys Leu Ala Gln Ala Gly Ala Tyr Gln Cys
    780                 785                 790

Ile Val Asp Asn Gly Val Ala Pro Pro Ala Arg Arg Leu Leu Arg Leu
795                 800                 805                 810
```

Val Val Arg Phe Ala Pro Gln Val Glu His Pro Thr Pro Leu Thr Lys
815 820 825

Val Ala Ala Ala Gly Asp Ser Thr Ser Ser Ala Thr Leu His Cys Arg
830 835 840

Ala Arg Gly Val Pro Asn Ile Val Phe Thr Trp Thr Lys Asn Gly Val
845 850 855

Pro Leu Asp Leu Gln Asp Pro Arg Tyr Thr Glu His Thr Tyr His Gln
860 865 870

Gly Gly Val His Ser Ser Leu Leu Thr Ile Ala Asn Val Ser Ala Ala
875 880 885 890

Gln Asp Tyr Ala Leu Phe Thr Cys Thr Ala Thr Asn Ala Leu Gly Ser
895 900 905

Asp Gln Thr Asn Ile Gln Leu Val Ser Ile Ser Arg Pro Asp Pro Pro
910 915 920

Ser Gly Leu Lys Val Val Ser Leu Thr Pro His Ser Val Gly Leu Glu
925 930 935

Trp Lys Pro Gly Phe Asp Gly Gly Leu Pro Gln Arg Phe Cys Ile Arg
940 945 950

Tyr Glu Ala Leu Gly Thr Pro Gly Phe His Tyr Val Asp Val Val Pro
955 960 965 970

Pro Gln Ala Thr Thr Phe Thr Leu Thr Gly Leu Gln Pro Ser Thr Arg
975 980 985

Tyr Arg Val Trp Leu Leu Ala Ser Asn Ala Leu Gly Asp Ser Gly Leu
990 995 1000

Ala Asp Lys Gly Thr Gln Leu Pro Ile Thr Thr Pro Gly Leu His Gln
1005 1010 1015

Pro Ser Gly Glu Pro Glu Asp Gln Leu Pro Thr Glu Pro Pro Ser Gly
1020 1025 1030

Pro Ser Gly Leu Pro Leu Leu Pro Val Leu Phe Ala Leu Gly Gly Leu
1035 1040 1045 1050

Leu Leu Leu Ser Asn Ala Ser Cys Val Gly Gly Val Leu Trp Gln Arg
1055 1060 1065

Arg Leu Arg Arg Leu Ala Glu Gly Ile Ser Glu Lys Thr Glu Ala Gly
1070 1075 1080

Ser Glu Glu Asp Arg Val Arg Asn Glu Tyr Glu Glu Ser Gln Trp Thr
1085 1090 1095

Gly Glu Arg Asp Thr Gln Ser Ser Thr Val Ser Thr Thr Glu Ala Glu
1100 1105 1110

```
Pro Tyr Tyr Arg Ser Leu Arg Asp Phe Ser Pro Gln Leu Pro Pro Thr
1115                1120                1125                1130

Gln Glu Glu Val Ser Tyr Ser Arg Gly Phe Thr Gly Glu Asp Glu Asp
                1135                1140                1145

Met Ala Phe Pro Gly His Leu Tyr Asp Glu Val Glu Arg Thr Tyr Pro
            1150                1155                1160

Pro Ser Gly Ala Trp Gly Pro Leu Tyr Asp Glu Val Gln Met Gly Pro
        1165                1170                1175

Trp Asp Leu His Trp Pro Glu Asp Thr Tyr Gln Asp Pro Arg Gly Ile
    1180                1185                1190

Tyr Asp Gln Val Ala Gly Asp Leu Asp Thr Leu Glu Pro Asp Ser Leu
1195                1200                1205                1210

Pro Phe Glu Leu Arg Gly His Leu Val
                1215
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer exon 2 5'UTR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GAGAAAGCCA GACAGACGCA G
21
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer intron 2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
AGCTTCCGCT GGTGGCT
17
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer intron 23"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCGGGGAGA CCCACCC
17

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer intron 26"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCTGATGCTA ACGGCAGGGC
20

WE CLAIM:

1. An isolated nucleic acid having the nucleic acid sequence of SEQ ID NO:1.

2. An expression vector containing the nucleic acid of claim 1.

3. An expression vector of claim 2 wherein said nucleic acid contains at least one intron.

4. An isolated protein encoded for by the nucleic acid of claim 1.

5. An isolated protein of claim 4 having the amino acid sequence of SEQ ID NO:2.

6. A method for detecting susceptibility to basement membrane disease, or the presence of existing basement membrane disease comprising detecting a mutation in a nephrin gene.

7. A method as in claim 6 comprising detecting a mutation in the nephrin protein.

8. A method as in claim 6 comprising detecting the presence or absence of nephrin protein.

9. A method as in claim 6 wherein said basement membrane disease is specifically congenital nephrotic syndromes of the Finnish type.

10. A kit for screening individuals for susceptibility to basement membrane disease, or the presence of basement membrane disease, containing at least one nucleic acid probe which detects the nucleic acid of claim 1.

11. A method for treating an individual with basement membrane disease comprising administering an effective therapeutic amount of a protein of claim 4.

12. A method for treating an individual with basement membrane disease comprising administering an effective therapeutic amount of nucleic acid constructs containing an expressible nucleic acid of claim 1.

13. A polyclonal antiserum containing antibodies specific for nephrin protein produced by immunizing an animal with a sufficient amount of the protein of claim 5 to stimulate an immune response.

14. A monoclonal antibody specific for nephrin produced by immunizing a rodent with a sufficient amount of the protein of claim 5 to stimulate an immune response, harvesting spleen cells from said immunized rodent, hybridizing said spleen cells with a suitable hybridoma partner, screening resultant hybridoma cells for said specific monoclonal antibody.

15. A chimeric antibody comprising the variable domains of the antibody of claim 14 functionally attached to human antibody constant domains.

16. A kit for screening individuals for susceptibility to basement membrane disease, or the presence of basement membrane disease, containing at least one antibody specific for nephrin.

17. A method for identifying a small molecule therapeutic for the treatment of proteinuria associated with kidney disease comprising screening candidate molecules for specific binding to the nephrin protein.

18. A method as in claim 17 wherein said specific binding effects a change in nephrin protein bioactivity.

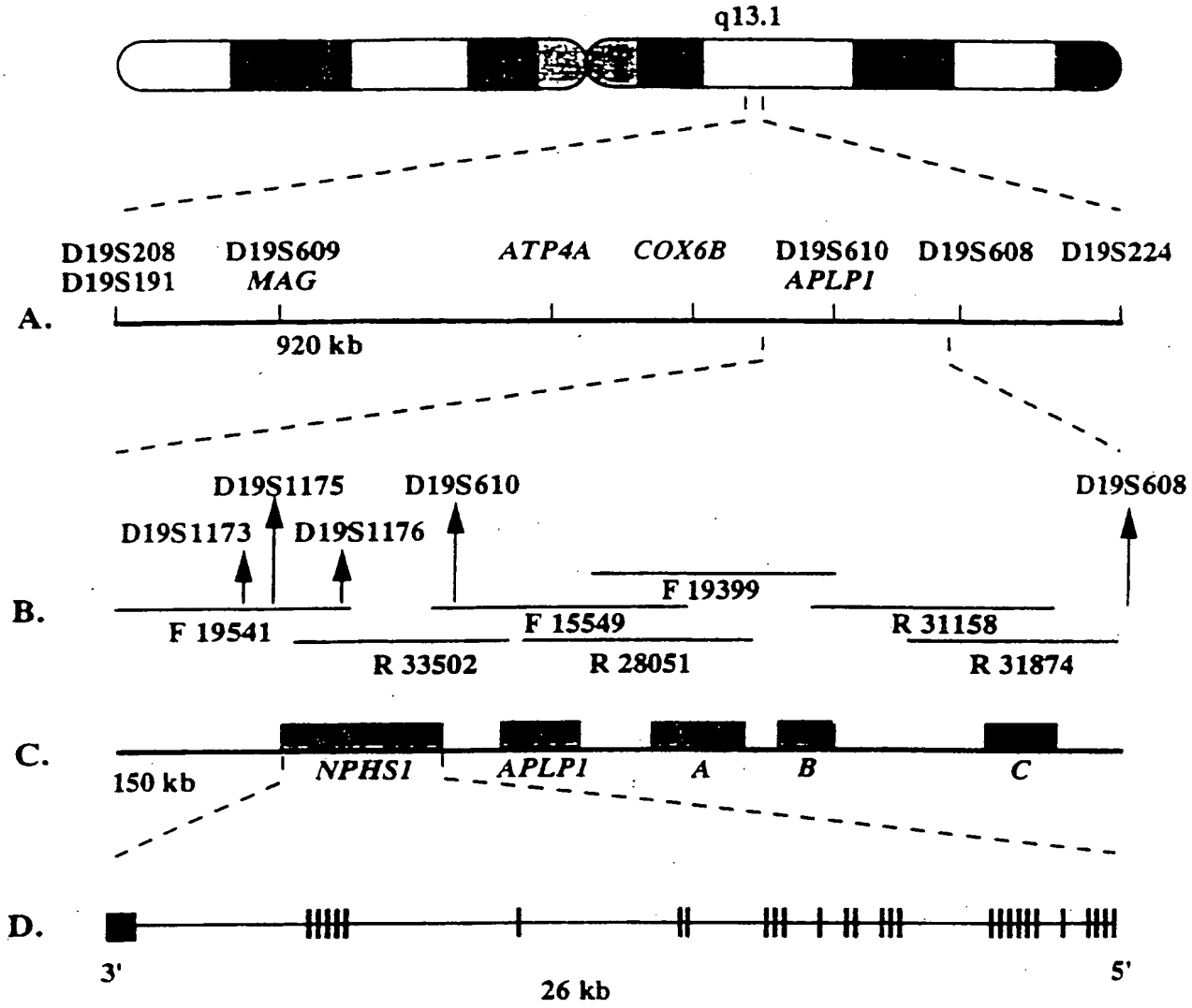
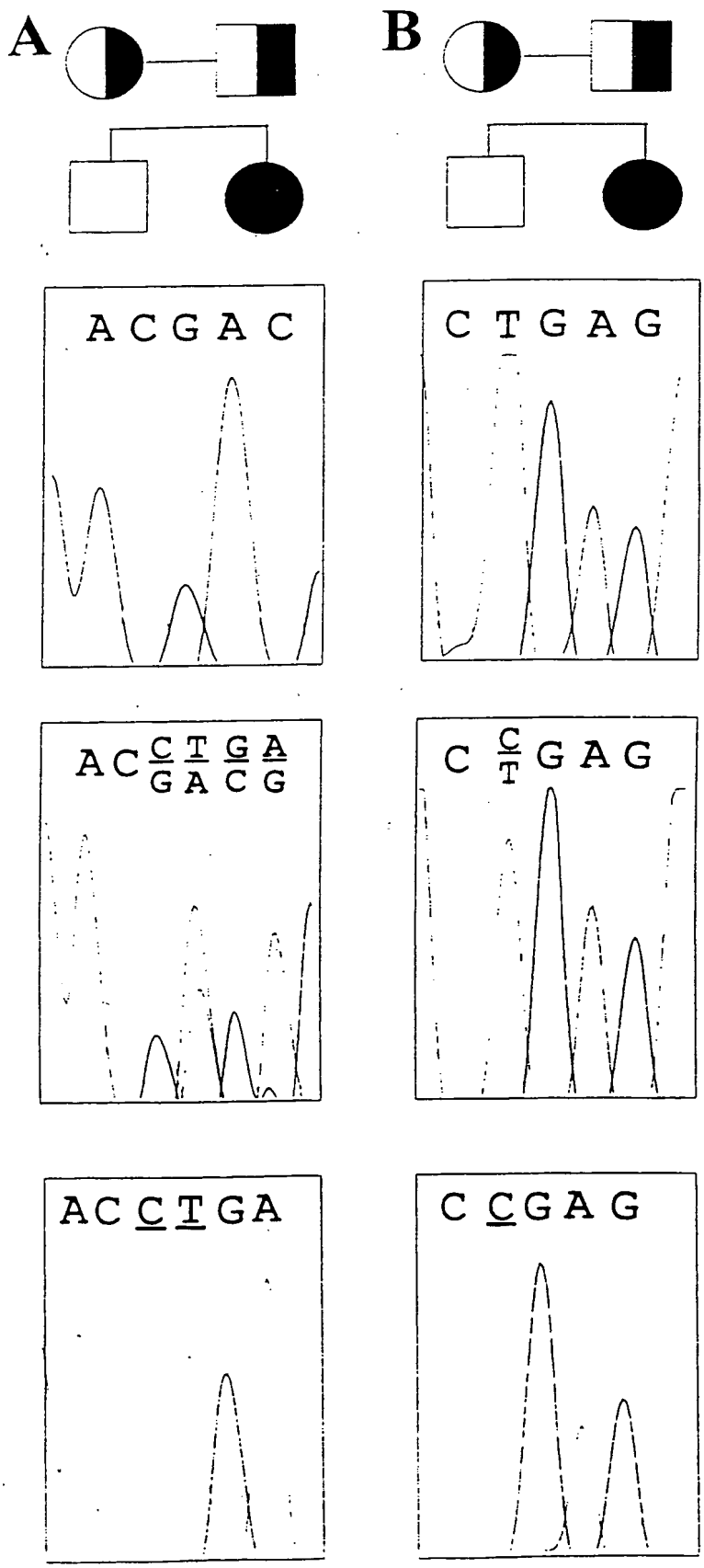

Chromosome 19
q13.1
D19S208
D19S191
D19S609
MAG
ATP4A
COX6B
D19S610
APLP1
D19S608
D19S224
A.
920 kb
D19S1175
D19S610
D19S608
D19S1173
D19S1176
B.
F 19399
F 19541
F 15549
R 31158
R 33502
R 28051
R 31874
C.
150 kb
NPHS1
APLP1
A
B
C
D.
3'
5'
26 kb

Fig. 1

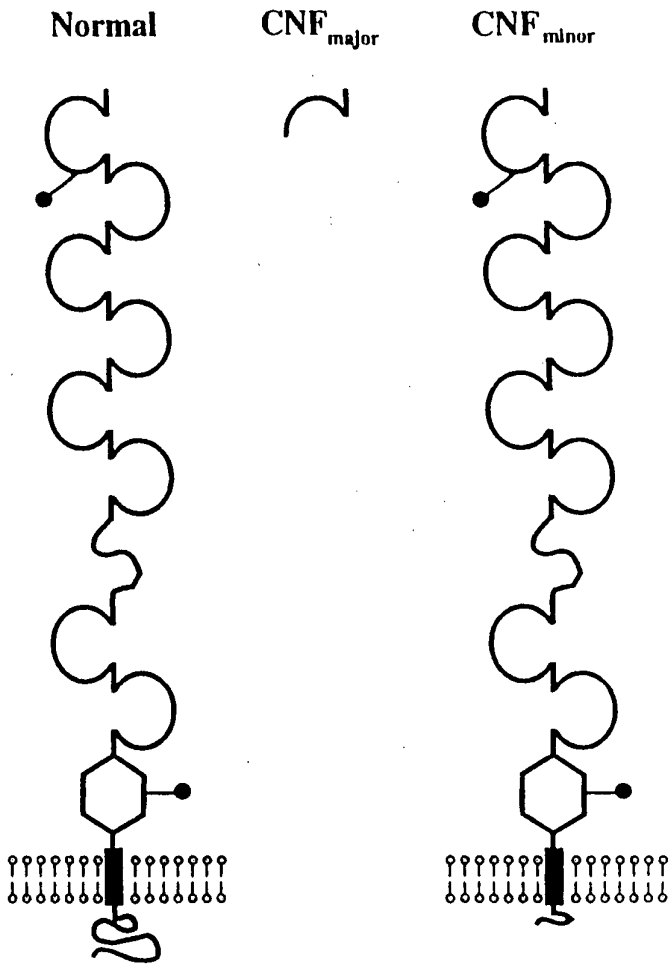
Fig. 2

Fig. 3


A
B
ACGAC
CTGAG
Patient
ACCTGA
GACG
CCGAG
Carrier parent
ACCTGA
CCGAG
Healthy sibling

```
1    MALGTTLRAS LLLLGLLTEG LAQLAIPASV PRGFWALPEN LTVVEGASVE
51   LRCGVSTPGS AVQWAKDGLL LGPDPRIPGF PRYRLEGDPA RGEFHLHIEA
101  CDLSDDAEYE CQVGRSEMGP ELVSPRVILS ILVPPKLLLL TPEAGTMVTW
151  VAGQEYVVNC VSGDAKPAPD ITILLSGQTI SDISANVNEG SQQKLFTVEA
201  TARVTPRSSD NRQLLVCEAS SPALEAPIKA SFTVNVLFPP GPPVIEWPGL
251  DEGHVRAGQS LELPCVARGG NPLATLQWLK NGQPVSTAWG TEHTQAVARS
301  VLVMTVRPED HGAQLSCEAH NSVSAGTQEH GITLQVTFPP SAIIILGSAS
351  QTENKNVTLS CVSKSSRPRV LLRWWLGWRQ LLPMEETVMD GLHGGHISMS
401  NLTFLARRED NGLTLTCEAF SEAFTKETFK KSLILNVKYP AQKLWIEGPP
451  EGQKLRAGTR VRLVCLAIGG NPEPSLMWYK DSRTVTESRL PQESRRVHLG
501  SVEKSGSTFS RELVLVTGPS DNQAKFTCKA GQLSASTQLA VQFPPTNVTI
551  LANASALREG DALNLTCVSV SSNPPVNLSW DKEGERLEGV AAPPRRAPFK
601  GSAAARSVLL QVSSRDHGQR VTCRAHSAEL RETVSSFYRL NVLYRPEFLG
651  EQVLVVTAVE QGEALLPVSV SANPAPEAFN WTFRGYRLSP AGGPRHRILS
701  SGALHLWNVT RADDGLYQLH CQNSEGTAEA RLRLDVHYAP TIRALQDPTE
751  VNVGGSVDIV CTVDANPILP GMFNWERLGE DEEDQSLDDM EKISRGPTGR
801  LRIHHAKLAQ AGAYQCIVDN GVAPPARRLL RLVVRFAPQV EHPTPLTKVA
851  AAGDSTSSAT LHCRARGVPN IVFTWTKNGV PLDLQDPRYT EHTYHQGGVH
901  SSLLTIANVS AAQDYALFTC TATNALGSDQ TNIQLVSISR PDPPSGLKVV
951  SLTPHSVGLE WKPGFDGGLP QRFCIRYEAL GTPGFHYVDV VPPQATTFTL
1001 TGLQPSTRYR VWLLASNALG DSGLADKGTQ LPITTPGLHQ PSGEPEDQLP
1051 TEPPSGPSGL PLLPVLFALG GLLLLSNASC VGGVLWQRRL RRLAEGISEK
1101 TEAGSEEDRV RNEYEESQWT GERDTQSSTV STTEAEPYYR SLRDFSPQLP
1151 PTQEEVSYSR GFTGEDEDMA FPGHLYDEVE RTYPPSGAWG PLYDEVQMGP
1201 WDLHWPEDTY QDPRGIYDQV AGDLDTLEPD SLPFELRGHL V
```

A



B

Fig. 4

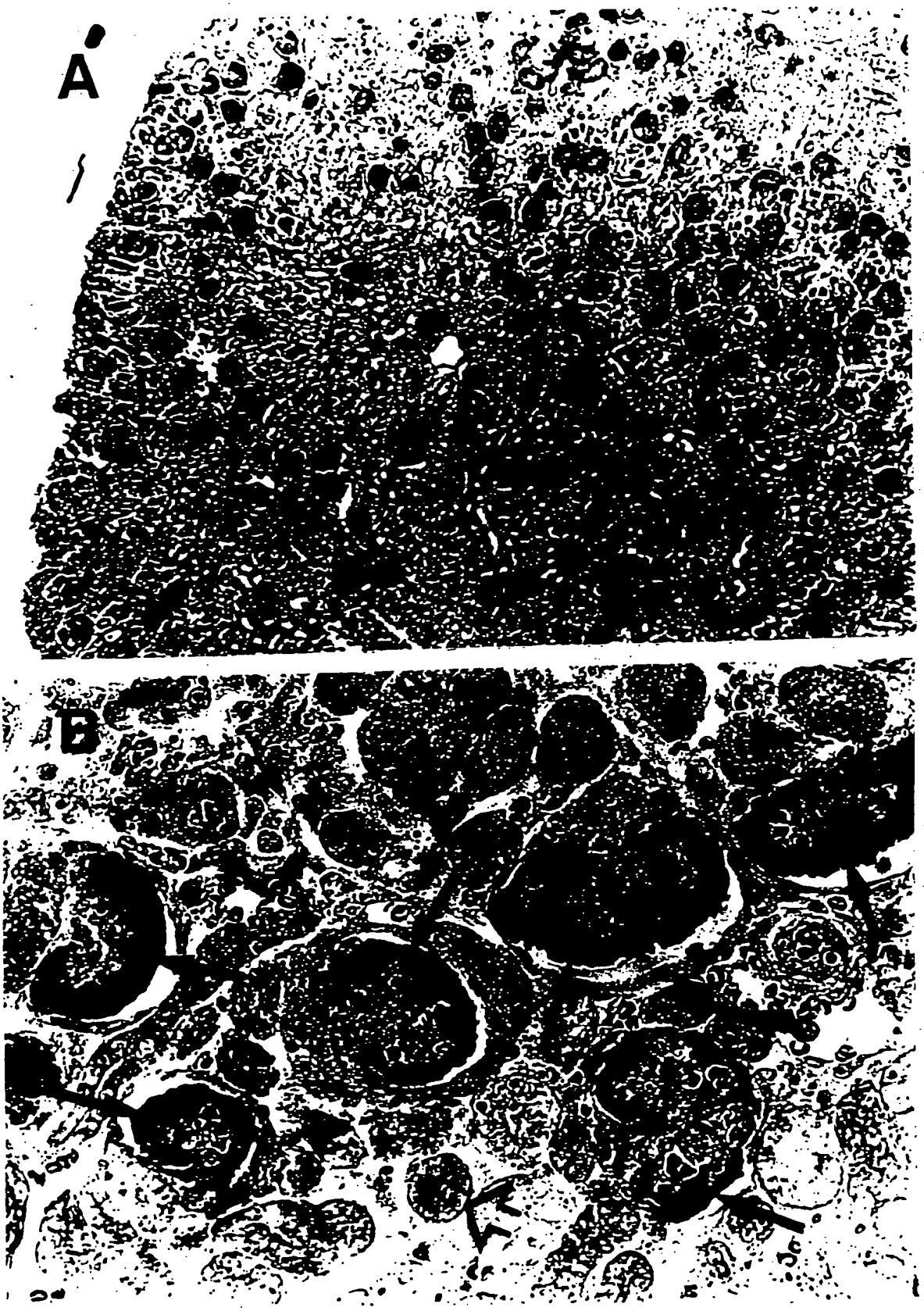


Fig. 5